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Electrostatic Spray Application of Food-Grade Organic Acids and Plant Extracts to Decrease Escherichia coli O157:H7 and Salmonella Typhimurium on Select Produce

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By

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ABSTRACT

Both consumers and suppliers have been negatively affected by an increase in foodborne pathogens contaminating fruits. Consequently, there is a need for the development of more efficient antimicrobials and application techniques to decrease contamination. Natural preservatives such as organic acids and plant extracts have demonstrated promising results in decontaminating produce. In addition, the effectiveness of such preservatives may be enhanced by the use of an electrostatic sprayer. The objective of this research was to determine the combinations and concentrations of organic acids and plant extract that were able to reduce *Salmonella* Typhimurium (ST) and *Escherichia coli* O157:H7 (EC) inoculated fruits and how the efficiency of the electrostatic spraying technique compares to conventional spraying. Quality attributes of the treated fruit were tested to determine if further deterioration occurred. Cantaloupe cubes and tomatoes were inoculated with ST and EC, and then electrostatically sprayed with different concentrations of organic acids and grape seed extract and stored at 4°C for 11 and 12 days, respectfully. Malic acid (M) (4%), alone and combined with lactic acid (L) (2%) demonstrated the greatest reduction of ST (reduced by 3.3 and 3.6 log CFU/g) and EC (4.6 log CFU/g) on cantaloupe cubes during the storage period. Lactic acid alone and in different combinations with M were able decrease EC (2.3 log CFU/g) and ST ($\geq$ 3.7 log CFU/g) on tomatoes after 12 days of storage. Compared to conventional spraying, electrostatic spraying of organic acids was more effective in reducing more EC and ST on cantaloupe cubes. Electrostatic sprayer reduced a significant amount of EC on tomatoes, but there was no difference between the types of sprayers when reducing ST. No significant differences in color were observed when comparing non-sprayed cantaloupe cubes and tomatoes to sprayed samples. The texture of cantaloupe left untreated was no different from cantaloupe cubes treated with organic acids.

Tomatoes sprayed with organic acids did not differ from untreated tomatoes until the 12^{th} day of storage. Natural antimicrobials have the potential to be used to enhance the safety of produce without compromising the quality. Usage of multiple hurdle technology in the produce can be used along with current practices to enhance the safety of food.

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Chapter 1: Introduction

Outbreaks of Foodborne Illness

Contaminated food is estimated to cause 48 million illnesses, 128,000 hospitalizations, and 3000 deaths per year (CDC 2011d). These outbreaks are caused by a long list of pathogens that contaminate meat products and produce (CDC 2011d). The outbreaks have been categorized into four groups that have caused approximately 51% of foodborne illness which are beef, poultry, produce, and seafood (DeWaal and Glassman 2013; Ukuku, 2004). There was a major outbreak of *Salmonella* Typhimurium that affected most of the US which was linked to peanut butter (CDC 2010). This outbreak was followed by two different outbreaks linked to beef that were contaminated with *Escherichia coli*O157:H7 in 2009 (CDC 2009a; CDC 2009b).

Fresh cut fruits and vegetables that are often eaten without processing or minimal processing are potential sources of foodborne pathogens. The outbreaks tied to produce are more often devastating because of high mobility rate, difficulty locating contaminated produce, and tracing the source of contaminate (Akkad 2005). These outbreaks can be devastating to the consumers and the companies that produce the products. Recently there has been an outbreak of *Listeria monocytogenes* that was linked to cantaloupe (CDC 2011c). This outbreak caused a recall of other possibly contaminated cantaloupes (FDA 2011).

Produce has also been linked to outbreaks of *Salmonella* spp. and *E. coli* O157:H7. The multistate outbreak of *Salmonella spp*. has been linked to alfalfa sprouts and cantaloupe. Approximately 140 individuals were infected with the pathogens in 26 states due to sprout consumption; also 13 people have been infected with *Salmonella* Panama in 5 states because of the consumption of cantaloupe (CDC 2011a; CDC 2011b). *Salmonella spp.* and *E. coli* O157: H7 have been able to cause problems because of their ability to survive in different environments.

Contamination and Sanitation of Produce

Contamination of fresh cut produce can occur during harvesting, processing, storage, handling, and/or during preparation, which can be from direct or indirect contact with fecal matter (Ukuku 2004; Pangloli *et al.* 2009). Most produce is grown on or near ground level, so the outer skin can become contaminated by irrigation water and manure fertilizer (Mahmoud *et al.* 2008). Another source of contamination of produce can be from contaminated seed that provide favorable conditions for the pathogen to flourish (Sharma *et al.* 2003). Produce has the potential to get contaminated throughout production and processing, and seed decontamination cannot solve the entire problem. A study showed no elimination of *Salmonella* Typhi and *Vibrio cholera* O1, inoculated on alfalfa seeds, when treated with antimicrobials like sodium hypochlorite, calcium hypochlorite, and hydrogen peroxide (Sharma *et al*. 2003; Pao *et al.* 2008). During processing and preparation, there can be cross contamination from the equipment and food handlers (Mahmoud *et al.* 2008). Most processors of fresh and fresh-cut produce perform a washing treatment (water and a sanitizer like chlorine). However, the chlorine in the water has the potential to produce harmful byproducts like trihalomethanes which have carcinogenic effects (Sagong *et al*. 2011).

Natural Antimicrobials

Organic acids have potential as antimicrobial agents because of their acceptance in food products, low cost, and GRAS (generally recognized as safe) status (Choi *et al*. 2009; Ganesh *et al*. 2010). Organic acids are found in fruits and vegetables and/or as the result of metabolic pathways. Many studies have been done on organic acids as antimicrobials on different food items including produce and have been found to be effective. Savard *et al.* (2002) used acetic acid, lactic acid, and propionic acid to reduce or inhibit spoilage yeasts (*Saccharomyces bayanus* and *Saccharomyces unisporus*) on fermented vegetables. The study found acetic (0.4%) and propionic (0.2%) acid in combination reduced *S. unisporus* and inhibited *S. bayanus*. Along with organic acids, plant extracts have shown promising results as antimicrobials. Extracts from vanilla, coriander, cilantro, lemon balm, oregano and mustard oil, grape seed and green tea extracts have been screened for antimicrobial properties (Choo *et al*. 2006; Gutierrez *et al.* 2009; Ganesh *et al.* 2010). These plant extracts have shown to reduce foodborne pathogens like *E. coli* O157:H7 and *Salmonella* Typhimurium on different food products. In one study, grape seed extract applied to tomatoes, inoculated with *Listeria monocytogenes*, was able to reduce pathogens by approximately 2 logs during two minutes of exposure (Bisha *et al.* 2010). Literature suggests that organic acids and plant extracts are promising antimicrobials for fresh and organic fruits and vegetables.

Antimicrobial Application

The application of organic acids and plant extracts on the fresh and fresh-cut fruits and vegetables is important because if the antimicrobials are not applied properly they cannot be very effective against target pathogens. In industry, sanitizing treatments are usually applied using dump tanks (dipping) or conventional sprayers (Parish *et al.* 2003; Alvarado-Casillas *et al.* 2007). The use of dump tanks has the possibility to promote internalization of pathogen if water temperature is not maintained or there can be cross contamination from other produce (Alvarado-Casillas *et al*. 2007). Conventional sprayers used to deliver sanitizing treatments use excess solution and deposit large droplets that cause run off (Electrostatic Spraying Systems 2011).

Research has been performed to investigate different strategies to apply natural antimicrobials. There has been much research done on incorporating organic acids and plant extracts in edible films to protect against pathogenic and spoilage organisms (Ouattara *et al.*

2000; Eswaranandam *et al.* 2004; Gadang *et al.* 2008). Electrostatic spraying has been used for pesticide and fungicide application on crops and production lines. This technique allows for smaller particles of solutions to deposit on the surface of the target (Law and Cooper 2001; Law 2001). Ganesh *et al*. (2010) used electrostatic spray to apply organic acids and plant extracts as a method of reducing pathogens on lettuce. In this study, researchers showed that antimicrobials applied with the electrostatic sprayer reduced more *Salmonella* Typhimurium than by conventional spraying, which deposits larger droplets that can runoff the surface (Ganesh *et al.* 2012). Since electrostatic spraying is more effective in applying antimicrobials, studies need to be performed to determine the reduction of pathogens in a variety of produce.

Quality Attributes

Quality attributes are important to consumers when choosing fresh fruits and vegetables. Two of the most important quality attributes to the consumer and industry are color and texture. It is important to conserve the quality of the food products when applying any kind of preservative or antimicrobial on fresh produce (Rosenfeld and Nes 2000; Eswaranandam *et al.* 2006). Texture and color analysis has been studied on fruits and vegetables that have been altered either by the addition of antimicrobials or preservation method (Chassagne-Berces *et al.* 2009). Palekar *et al.* (2004) found that the color and texture attributes of cantaloupe were not affected when treated with irradiation. In another study, apples that were frozen at different temperature and thawed were analyzed texturally. The results showed that apples frozen at -80°C had better textural quality than the other freezing method; however, the frozen-thawed apple was still significantly different from a regular unthawed apple (Palekar *et al.* 2004). Therefore, testing the texture and color of the fruits and vegetables that are treated with antimicrobials is very important to make sure there are no adverse effects.

With the continuous foodborne outbreaks, more efficient techniques on reducing pathogens contamination are needed. The different antimicrobials have been studied to prevent further outbreaks. New technology along with natural antimicrobials when used on fresh produce can have an impact on reducing contamination and improving commercial food safety.

Objectives

The objectives are to:

1. Investigate the effects of electrostatic spray of malic acids, lactic acid, and grape seed extract at 0, 1, 2, 3, and 4% on inoculated surfaces of cantaloupe and tomatoes at 4 °C.
2. Investigate the effect of electrostatic spray containing optimized concentration of antimicrobials on the color and texture of uninoculated cantaloupe and tomatoes.

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Chapter 2: Literature Review

Outbreaks and Recalls of Foodborne Illness

The idea of a healthier lifestyle has led consumers to make healthier choices in their diet. These choices have led to an increased demand for fresh fruits and vegetables. The products are usually consumed without processing. Without processing, the consumers are at risk for becoming infected with foodborne pathogens that may have contaminated produce (Berger *et al.* 2010). Between 1999 and 2008, produce was linked to largest number of foodborne illnesses (DeWaal *et al.* 2010).

Contaminated food in the United States has led to approximately 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths annually. Most foodborne infections cause gastrointestinal problems, but if an immunocompromised person is infected they can develop bloodstream infection, kidney failure, miscarriages, and other life threatening symptoms. The infection that has increased over the years has been salmonellosis. Illnesses caused by *E. coli* O157:H7 has decreased from 1996 to 2006 when compared to the incidence of salmonellosis. However, there has been no significant difference in infections caused by *E. coli* from 2006-2010 (CDC 2011e).

Salmonella is a rod shaped, gram negative, non-spore forming bacteria that are the most frequently cause of foodborne illnesses. The pathogen causes salmonellosis with symptoms that include diarrhea and abdominal cramps 12 – 72 hours after infection (Berger *et al.* 2010). Foodborne illnesses caused by *Salmonella* have been linked to the consumption of tomatoes, melons, sprouts, poultry, peanuts, and other food products. The most common serotypes in the United States are Typhimurium and Enteritidis. There are around 400,000 cases of salmonellosis every year. However, many milder cases go undiagnosed and unreported, so the number of infections could be greater (CDC 2010b).

In recent years, there has been an increase in outbreaks of salmonellosis. In March 2011, an outbreak of *Salmonella* Panama infections was linked to cantaloupe, with a total of 20 people being infected in 10 states (CDC 2011c). In July 2011, a second outbreak of *Salmonella* was linked to alfalfa sprouts and spicy sprouts. A total of 25 people have been infected with this pathogen. Because of the outbreak, the company that sold the alfalfa sprouts recalled specific lots of sprouts (CDC 2011b). More recently, there has been an outbreak of *Salmonella* Newport and Typhimurium linked to the consumption of cantaloupe. This outbreak has caused 270 people to become ill, 101 of them were hospitalized, and 3 died. Like the outbreak linked to alfalfa sprouts, cantaloupes have been recalled from the farm the pathogen was traced back to (CDC 2012b).

Escherichia coli is a gram negative, rod-shaped foodborne pathogen. There are 6 different serotypes: enteroaggregative, enteroinvasive, enteropathogenic, enterotoxigenic, diffuse adherent, and enterohemorrhagic. Enterohemorrhagic *E. coli* O157:H7 is usually connected to foodborne outbreaks and the most common. This serotype is the causative agent of severe gastrointestinal disease in humans and is responsible for most cases of HUS (hemolytic uremic syndrome). This pathogen has been linked to spinach, sprouts, meat products, and other food items (Silagyi *et al*. 2009). It was first identified as a pathogen in 1982 outbreak traced to hamburgers from fast food chain (Tauxe 1997). There has been an estimated 265,000 infections of *E. coli* each year in the U.S, but 36% of these are caused by O157:H7 pathogens. The largest outbreak of *E. coli* O157:H7 was in 2006 with the contamination of spinach. This pathogen infected 199 people in 26 different states. Some of the infected people developed HUS which affects the kidneys and can lead to death (CDC 2011a).

There has been no change in the number of O157 infections per year, but there have been other outbreaks of *E. coli* serogroups that have been linked to fruits and vegetables (Ferguson 2005; CDC 2006; CDC 2010a; CDC 2011d). In 2010, there was an outbreak of *E. coli* O145 that was linked to shredded romaine lettuce. A total of 26 cases were reported from 5 states. Of the 26 cases, 3 cases developed HUS, but no deaths have been reported (CDC 2010a). The most recent outbreak in Germany was caused by *E. coli* O104, and linked to the consumption of sprouts. This particular *E. coli* was also linked to 6 cases in the United States. There was one death in Arizona, in which the resident traveled to Germany before becoming ill (CDC 2011d). Romaine lettuce was the source of a recent outbreak of *E. coli* O157:H7 (CDC 2012a). The outbreak affected 60 people in 10 states. Among affected persons, only 2 people developed HUS and there were no deaths. Outbreaks associated with the consumption of sprouts can be due to contaminated seeds or sprouts. The conditions which sprouts are grown are ideal for the elevating the growth of pathogens (Mandrell 2009).

These foodborne pathogens have different modes of entering the human system. *Salmonella* and *E. coli* can attach to surface of produce and when the produce is ingested the pathogen is free to cause problems. To prevent these occurrences, proper sanitation needs to be performed.

Sanitation of Produce

There are many techniques to reduce the population of microorganisms on the surface of fresh and fresh-cut produce. The antimicrobials can vary in effectiveness due to chemical, physical, and mechanical action during the application process (Herdt and Feng 2009). Physical treatments involve the use of brushes to remove soil and microorganisms. However, this treatment can only be used to on “hardy” produce. Produce that can be damaged by brushes are usually washed in a bath or treated under a spray (Parish *et al.* 2003).

Fresh produce is usually washed in a chemical/sanitizing treatment. The most widely used chemical treatment is chlorine and chlorine compounds (Parish *et al.* 2003; Sapers *et al.* 2006). The common forms of chlorine for washing are liquid chlorine and hypochlorite. Liquid chlorine and hypochlorite are used at a concentration of 50 to 200 ppm with contact time of 1-2 minutes (Parish *et al.* 2003). There has been mixed results in determining the effect of chlorine on pathogen contaminated on surface of produce (Parish *et al.* 2003). Studies on alfalfa sprouts and seed treated with 100 ppm of chlorine reduced *Salmonella* populations; however with higher concentration, no further reduction was observed (Jaquette *et al.* 1996). In another study, alfalfa seeds and sprouts inoculated with *Salmonella* were treated with 500 ppm chlorine dip that led to a reduction of 3.4 log per gram of the pathogen. At a higher concentration (2000 ppm), *Salmonella* population was undetectable (Beuchat and Ryu 1997; Parish *et al.* 2003). However, the highest concentration allowed for chlorine to sanitize fresh fruits and vegetable is 200 ppm (Huang and Chen 2011). *Salmonella* Montevideo inoculated on tomatoes recovered after being stored up to 10 days after being treated with chlorine (200 ppm and 1000 ppm). The maximum reduction seen was on day 0 (4.8 log CFU) (Iturriaga and Escartín 2010).

Chlorine has a disadvantage of reacting with organic matter which may neutralize chlorine or may lead to the production of harmful byproducts such as trihalomethanes (Parish *et al.* 2003; Sapers *et al*. 2006). Trihalomethanes have been linked to cancer, miscarriages, and birth defects (Huang and Batterman 2009). Along with the formation of harmful by products, another limitation of chlorine is rapid depletion with increasing organic load, required pH adjustment to solution, and off-gassing during processing (Herdt and Feng 2009). Therefore, studies have been pursued to find better alternatives to chlorine. Different antimicrobials like hydrogen peroxide, ozone, and other chemicals have been studied as alternatives to chlorine as antimicrobials for produce.

Hydrogen peroxide is a GRAS chemical that can be used in food products as a bleaching, oxidizing and reducing, and antimicrobial agent. The FDA has approved the antimicrobial to be used on minimally processed produce unless it is combined with acetic acid to form peroxyacetic acid. In numerous studies, hydrogen peroxide has been shown to be an excellent antimicrobial agent. In one study, spinach inoculated with *E. coli* O157:H7 was treated with hydrogen peroxide and a mild heat (50°C) to yield a reduction 2.2 log CFU/g at 2% concentration (Huang and Chen 2011). Ukuku (2004) found that hydrogen peroxide was able to reduce *Salmonella* population on whole cantaloupe by approximately 2.6 logs. However, when the concentration was increased no significant increase in reduction was observed.

Ozone has been effective in reducing human pathogens in water, and ozonated water has shown to reduce microbial population on apples and berries to extend shelf life. Herdt and Feng (2009) reported a study that found a 4 log reduction of *E. coli* O157:H7 after exposing lettuce to 3mg/L of ozone for 3 min. However, there are not many studies that demonstrate ozone's effectiveness as a pathogen reducer on produce (Parish *et al.* 2003; Sapers et al. 2006). A drawback of using ozonated water is the potential for off-gassing and decrease effectiveness due to organic levels in the water wash (Herdt and Feng 2009).

Organic Acids

Organic acids occur naturally. These acids are found in fruits and vegetables and/or a result of biological pathways. Organic acids are promising antimicrobial agents because of their acceptance in food products, low cost, and have a GRAS (generally recognized as safe) status

(Choi *et al.* 2009; Ganesh *et al.* 2012). Malic acid, citric acid, lactic acid, and acetic acid are a few of the organic acids that have been used in research to protect against foodborne pathogens. Malic acid is found in apples and some grapes (Jensen 2007). Lactic acid is the byproduct of microbial fermentation (Dumbrepatil *et al.* 2008).

Studies have been done to determine organic acid effectiveness and mechanisms by which the acids work. The mechanism by which organic acids work is the undissociated molecules enter the cell membrane and get ionized. The acidic pH in the cell triggers deformation and damage to the protein and DNA structure and enzymatic activity (In *et al*. 2013; Brul and Coote 1999). The damage can lead to membrane disruptions, reduction in metabolic reactions, accumulation of toxic anions and protons, and regulation pH homeostasis inside the cell (Ricke 2003; Gyawali and Ibrahim 2012). These acids have been found to reduce foodborne pathogen that have attached to the surface of food products. In a study, researchers immersed organic lettuce inoculated with *S.* Typhimurium in malic, lactic, and citric acid (Sagong *et al.* 2011). The results showed malic acid, lactic acid , and citric acid independently reduced the pathogen between approximately0.4 log CFU/g to approximately1.7 log CFU/g, depending on the concentration. An increase in the organic acid concentration (concentrations were in ranges of 0.3% to 2.0%) resulted in an increase in pathogen reduction (Sagong *et al.* 2011). Lactic acid combined with hydrogen peroxide was able to reduce *E. coli*, *Salmonella* Enteritidis, and *Listeria monocytogenes* to undetectable levels (50 CFU/fruit) after 15 minute of exposure (Venkitanarayanan *et al*. 2002).

Malic and lactic acids have been applied to different foods systems using numerous techniques. In two studies, the food grade organic acids have been incorporated into edible films as antimicrobials against *Salmonella spp.*, *E. coli* O157:H7, and *Listeria monocytogenes*

(Eswaranandam *et al.* 2004). The films in the first study were made from soy protein incorporated with citric, malic, lactic, and tartaric acids (0% and 2.6%) with and without nisin. The results showed that *S.* Gaminara was more suspectible to organic acids than *E. coli* O157:H7 and *Listeria monocytogenes*. The treatment with malic and lactic acid at 2.6% with nisin was able to reduce *S.* Gaminara by 5.7 and 3.4 log CFU/mL. However, films with malic acid (2.6%) and no nisin were able to reduce *S.* Gaminara *a* (3.0 log CFU/mL), E. *coli* O157:H7 (6.8 log CFU/mL), and *Listeria monocytogenes* (5.5 log CFU/mL). On another note, *E. coli* was also suspectible to lactic acid (2.6%) with and without nisin, however the treatment with nisin yielded a greater reduction (7.3 log CFU/mL compared to 7.0 log CFU/mL) (Eswaranandam *et al.* 2004). Another study was done with coatings (whey protein isolates that incorporated nisin, grape seed extract, malic acid, and EDTA) on turkey frankfurters that were inoculated with *E. coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* (Gadang *et al.* 2008). Results show that the *E. coli* and *Salmonella* Typhimurium populations were reduced by the coating containing 1%, 2%, and 3% malic acid alone. Even though malic alone was able to reduce the pathogens, the best treatment for *E. coli* O157:H7 was malic acid in combination with other antimicrobials. The treatment with nisin, malic acid, and EDTA reduced the most *E. coli* by 2.2 logs (Gadang *et al.* 2008). The use of electrostatic spray to apply organic acids to vegetables was used in a study, and showed promising results. Ganesh *et al.* (2010) found that lactic acid alone was able to reduce *Salmonella* Typhimurium by 1.5 log CFU/g for each day of observation and malic acid reduced the pathogen by 1.2 log CFU/g on the day 1 and 2.0 log CFU/g on day 7. Also in this study, it was found that high concentration of malic and lactic acid combined (3%) increased reduction of *Salmonella* Typhimurium from 1 log to 2 logs CFU/g (Ganesh *et al.* 2010).

Plant Extracts

Plant extracts from vanilla, coriander, cilantro, lemon balm, oregano and mustard oil, grape seed and green tea have been screened for antimicrobial properties (Choo *et al.* 2006; Gutierrez *et al.* 2009; Ganesh *et al.* 2010). Plant extracts are good alternatives as antimicrobials because they are less expensive and have GRAS (generally recognized as safe) status. The use of plant extracts has other advantages in addition to antimicrobial properties. Plant extracts like green tea and grape seed extract have antioxidant and anti-carcinogenic properties. Grape seed and green tea extracts have flavonoids that scavenge free radicals and have metal chelating properties (Perumalla and Hettiarachchy 2011).

Plant extracts have shown to reduce different foodborne pathogens. Ahn *et al.* (2007) reported that pine bark extract (Pyciongenol®) was most effective in inhibiting *Salmonella* Typhimurium and *E. coli* O157:H7. The extract was able to reduce the two pathogens by more than 1 log CFU/g (Ahn *et al.* 2007). Hop extract at different concentrations was used in coleslaw as antimicrobials against *Listeria monocytogenes*. This study showed that the highest concentration (1000 µg/mL) of hop extract was able to decrease *Listeria monocytogenes* during each day of observation (Larson *et al.* 1996). In one study, low concentration of grape seed extract (GSE) was used to reduce *Listeria monocytogenes* inoculated on tomatoes (Bisha *et al.* 2010). GSE (1250 µg/mL) reduced the pathogen by approximately 2 logs during two minutes of exposure. Green tea extract and grape seed extract were used in broth culture models and were able to reduce *Salmonella*, *E. coli*, and *Listeria monocytogenes* after 24 hr of exposure (Over *et al.* 2009).

Applying plant extracts and organic acids has been done mostly by dipping or immersion. Akbas and Ölmez (2007) were able to reduce *E. coli* and *Listeria monocytogenes* on lettuce by dipping samples in organic acid was treatments. In another study, poultry skin and lettuce were immersed in levulinic acid and SDS (sodium dodecyl sulfate) combination treatments that was able to reduce *Salmonella* and E. coli O157:H7 to undetectable levels (Zhao *et al*. 2009). However, immersion or dipping consumes a great deal of the antimicrobial treatment. Finding alternative methods to applying antimicrobials has been done and the use of hurdle technology has shown some promise.

Hurdle Technology

Hurdle technology is the use of different processes and technologies to improve the quality of a product. For food safety, hurdle technology has been studied and used in industrial settings. Delivery systems for organic acids and plant extracts have been combined with different preservation methods (storage temperatures, heat treatments, other antimicrobials) and technologies like ultrasound and edible films to reduce pathogenic microbes contaminated on food products (Perumalla and Hettiarachchy 2011).

To inhibit *Listeria monocytogenes* inoculated on turkey frankfurters, Sivarooban *et al.* (2007) added grape seed extract and nisin to the frankfurter formula. The combination had an immediate effect of the pathogen, and by the 21st day of the trial *Listeria monocytogenes* numbers were below minimum detection levels (Sivarooban *et al.* 2007). Another study used organic acids and hydrogen peroxide on baby spinach after different heat treatment (22°C, 40°C, and 50°C). The best treatment was lactic acid which had the highest reduction *E. coli* O157:H7 at all three temperatures (Huang and Chen 2011). Research was done on lettuce surfaces treated with different organic acids (acetic, citric, lactic, and propionic acid) at concentrations of 0.5% and 1.0% and stored at different temperatures to reduce *Listeria monocytogenes*. Three of the four organic acids used were able to reduce the pathogen level significantly when compared to the control (water). However, the organic acid to reduce the microbe to undetectable levels was lactic acid at concentration of 1% (Samara and Koutsoumanis 2009).

Different technologies can be used along with organic acids and plant extracts to enhance the effectiveness. One study used ultrasound along with organic acids to reduce *Salmonella* Typhimurium, *E. coli* O157:H7, and *Listeria monocytogenes* on organic lettuce. When treated with organic acids alone, the maximum reduction was approximately1.8 log CFU/g. Yet when treated with ultrasound waves after organic acids, the log reduction almost doubled (Sagong *et al.* 2011). Another study incorporated plant extract and other antimicrobials into an edible film to reduce pathogens. When grape seed extract, nisin, and EDTA were incorporated in soy protein edible films, the films were able to reduce *Salmonella* Typhimurium and *E. coli* levels by approximately 1 log (Sivarooban *et al.* 2008). Overhead sprayer equipped with rolling brushes demonstrated more reduction of *Salmonella* (4.0 log CFU/mL) from the surface of tomatoes than flume system (1.3 log CFU/ml) (Chang and Schneider 2012). Ganesh *et al.* (2010) combined grape seed extract along with malic acids and lactic acid to reduce *Salmonella* on spinach. The treatments were applied using an electrostatic sprayer and conventional sprayer. Malic acid in combination with grape seed extract showed a consistent progression of reduction when compared to the controls. However, the electrostatically sprayed malic acids and grape seed extract treatment showed better reduction (2.6 log and 3.3 log reduction on days 7 and 14) than conventionally sprayed treatment (0.5 log and 1.8 log reduction on days 7 and 14).

The electrostatic sprayer has shown the ability to deliver antimicrobial to contaminated products better than the conventional sprays. The ability of this technology to distribute antimicrobials needed to penetrate coat the produce and reduce pathogen existence should be further studied.

Electrostatic Spray

Electrostatic spray technology has been used in numerous commercial, business and industrial sectors (Law 2001). The spray technology has been used to distribute pesticides to protect field crops and orchards and also postharvest to prevent spoilage of stored fruits and vegetables (Law and Cooper 2001). The electrostatic sprayer works by having air and liquid enter the rear of the nozzle separately. The air and liquid meet at the nozzle tip. With the air under pressure, it causes the liquid to spray in smaller droplets. At the tip of the nozzle there is a tiny electrode that applies a charge to the spray. The charge allows the natural force of attraction between the stream of spray and target surface (Electrostatic Spraying Systems 2011).

Conventional sprayers or pressure sprayers use gravitational and inertial forces to deposit hydraulically atomized droplets. These sprayers have poor surface coverage, inefficient droplet deposition, and excessive rebound and runoff of spray liquid. In electrostatic spray technology, particles, charged and smaller than 100 μm diameter, are electrostatically deposited on the surface of produce (Kim and Hung 2007). The electrostatic spray method reduces wastage by being able to apply half the amount of solution on a subject and getting to the same protection as conventional spray method that uses the full solution (Law and Cooper 2001).

Research has been done to validate electrostatic spray technology whether used as various field machines and/or handheld devices. In one study, strawberry fields were sprayed with reduced-volume droplets (conventionally and electrostatically) of pesticide. It was found that all reduced-volume applications offered improved pesticide deposition and retention over time. However, charged, reduce-volume droplets significantly increased the initial deposition rate, and when only 50% of pesticide was applied, deposition and retention was equivalent to conventional, full rate application (Giles and Blewett 1991). Similar results were found in banana inoculated with fungal spores sprayed with an electrostatic sprayer and conventional sprayer with fungicide. The conventional spray applied a full-rate of chemical, and the electrostatic sprayer applied slightly less than half the full-rate of chemical. At half-rate chemical applications, approximately 6% exhibited crown rot compared to 20% crown rot on the conventional sprayed bananas (Law and Cooper 2001).

Using the electrostatic sprayer looks like promising deliver system for antimicrobials on vegetable and produce. However, research has not been done on how the sprayer affects the quality attributes of food products.

Quality Attributes of Produce

Sensory attributes are the most important factors for assessing the quality of food products. Two of the important quality attributes are color and texture (Rosenfeld and Nes 2000). Handling of the product possesses special problems because consumers have opinions and expectations of proper texture and color (Varela *et al.* 2007). Antimicrobial applications can have an effect on quality attributes. What the industry tries to accomplish when applying antimicrobials is to reduce the amount of contamination on the food product while conserving the quality (Rosenfeld and Nes 2000; Eswaranandam *et al.* 2006). Organic acids and plant extracts are used in the food industry as acidulants, flavor enhancers, antioxidants, and preservatives (Eswaranandam *et al.* 2006). To study the effect of organic acids and plant extract on food products, different techniques are used depending on the type of food.

Texture

Textural qualities have been studied using different methods. One study used a texture analyzer with 50 mm cylindrical cell to compress frankfurters which had locust bean/xanthan gum added and replaced pork fat with olive oil. The analyzer recorded force-time curves at

1mm/s. For the frankfurter with different concentrations of locust bean/xanthan gum, the texture profile showed that there was no significant change in hardness, gumminess, springiness and chewiness when compared to the control (no locust bean/xanthan gum). However when olive was added there was a significant decrease in many textural attributes (Lurueña-Martínez *et al.* 2004). Another study used the Kramer shear test to measure maximum shear force and total energy need to slice through beef products (ground beef and cook cured and uncured beef) with peanut skin extract. For the ground beef sampled, there was a significant increase in shear force (higher shear force for ground beef with 400 ppm of peanut skin extract), but there was not a significant increase in shear force (O'Keefe and Wang 2006). These texture analyzing methods are good techniques for meat products. However there are other methods that would be better suited for fruits and vegetables.

Texture analysis for fruits and vegetables is usually measured by a puncture and compression test. Puncture test measures the force needed to push a probe into the sample (Bourne 2002). This test method is used mostly to estimate harvest maturity or post-harvest inspection (Lu and Abbott 2004; Abbott 2004). There are different types of compression tests that are used on fruits and vegetables. There is a compression test that is uniaxial compression where force is applied to one end of the sample and it is allowed to expand freely. Uniaxial compression tests are commonly used for cylindrical samples taken from the fruit or vegetable (Lu and Abbott 2004). Using compression and puncture tests, researchers found that freezing apples at -80°C showed less degradation to apple firmness than other freezing methods (-20°C and liquid nitrogen immersion). Even though the apples frozen at -80°C were the most firm when compared to the other apples that were frozen by liquid nitrogen and at high temperature, when compared to the control the texture differences were very high (Chassagne-Berces *et al.*

2009). In another study, Varela *et al.* (2007) examined the changes in apple quality due to different storage times using uniaxial compression and puncture test. Both tests exhibited a loss of structural integrity as the storage time increased. However, the puncture test results did not show significant differences in structural integrity between day 7 and day 14. Uniaxial compression was able to show a significant difference in integrity between day 7 and day 14. This indicates that uniaxial compression is more sensitive to textural changes in the apple (Varela *et al.* 2007). The limitation to using a puncture test is the different probe. When using this method, researchers have to make sure they are using the appropriate probe depending on the surface of the food sample (Abbott 2004). Limitations to uniaxial compressions are the force applied and the plunger used to apply the force. Researchers have to make sure the plunger does not cut into the sample or shear forces will have to be accounted for (Bourne 2002).

The other compression method is compression extrusion where a force in applied to the food sample until it flows out of the outlet (Lu and Abbott 2004). The instrument used to measure the maximum force is Kramer shear press. This instrument passes a set of blades through a sample until the sample breaks and extrudes out of an opening. This method incorporated compression, shear, and extrusion. Cantaloupes and tomatoes have been tested using this instrument and method. The texture of fresh cut cantaloupe was measure by Kramer shear press after chemical treatment and vibrations were applied. The study reports that firmness of the treated cantaloupes decreased as throughout the experiments. This suggests that vibration can effects the quality of fresh-cut cantaloupe (Saha *et al.* 2009). Whole fresh market tomato firmness was tested by modified hear press and sensory analysis to determine if there was a correlation. The researchers found that mechanical means of texture analysis and sensory analysis has a rank correlation coefficient of 0.988, which shows a strong relationship between the two analyzes (Gormley and Keppel 1976; Barrett *et al.* 1998).

Color

Color analysis has been tested with hand-held colorimeter or chromameter. The colorimeter measures L*, a*, b* values. The L* values determines how much white or black is in the color with maximum value of 100 (white) and minimum value of 0 (black). The a* value determines how red or green the color is. The b*value determines how blue or yellow the color is. a* values and b* values are measured using the positive and negative numbers. Negative a* values are more green, and negative b* values are more blue in color. Positive a* values are more red in color, and positive b* values are more yellow (HunterLab 2008). Color is an important quality for freshness because consumers buy and eat with their eyes.

Color analysis is a factor that is tested to ensure that quality is not compromised when changes/improvements are made to produce. Sagong *et al.* (2011) used a colorimeter to measure the color change of fresh organic lettuce treated with ultrasound after an organic acid immersion. The results showed that no significant change in lettuce color after antimicrobial treatment (Sagong *et al.* 2011). Ukuku *et al.* (2006) monitored the color change of cantaloupe when treated with VSV (vacuum-steam-vacuum). Researchers found that VSV preserved the lightness, indicated by the L* value, of the cantaloupe during post-treatment storage. However, the a* values of samples stored at 10°C had decreased by day 6. This shows that cantaloupe held at this temperature had a shorter shelf life because even the control had a decreased a* value (Ukuku *et al.* 2006). These techniques of estimating texture and color changes can be used to determine if there were adverse effects to using hurdle technology.

Hurdle technology was used on sliced cantaloupe to reduce bacterial load and extend shelf life. The technology used was chlorine decontamination, irradiation, and modified atmospheric packaging. Texture and color attributes are monitored to determine which treatment had less effect on quality. Organic acids were used to reduce *Listeria monocytogenes* on romaine lettuce. Only three of the four organic acids used were able reduce the pathogen significantly when compared to the control (water). Different concentrations, storage temperatures, and times were used; and lactic acid was the only organic acid to continue to decrease the microbial load for all three parameters. Color attributes were tested to make sure there were not any undesirable affects from the organic acids. The treatments followed the same trend as the control (Samara and Koutsoumanis 2009). Results show that the multi-hurdle technology was able to reduce the bacterial load on the sliced cantaloupe for more than 10 days. However, the texture was significantly affected by the increase in irradiation (over 0.7 kGy). The color attributes were generally unaffected by the use of multi-hurdle technology (Palekar *et al.* 2004). Eswaranandam *et al.* (2004) demonstrated that malic and lactic acid-incorporated soy protein coatings were able to reduce *Salmonella* Gaminara, *E. coli* O157:H7, and *Listeria monocytogenes*. A follow up to this study was researching application of the soy coating on fresh cut fruit and testing texture and color qualities. The quantitative testing was compared to sensory panel test. The results showed there was no significant difference in texture or color when analyzing by mechanical means or by sensory testing (Eswaranandam *et al.* 2006). Testing color and texture of fresh produce after using hurdle technology is needed to make sure the quality is not compromised for safety.

Natural antimicrobials like organic acids and grape seed extract have been used in the food industry as preservatives. Certain GRAS status antimicrobials that are used in the industry have to have an addition rinse after usage. Using organic acid and plant extracts as antimicrobial can potentially remove the additional rinsing step so the antimicrobials have more contact time on the food surface. Along with using the organic acids and grape seed extract, the novel technology (electrostatic sprayer) has the potential to enhance the effectiveness of the antimicrobials. Quality tests are need to determine whether the natural antimicrobial cause deterioration to the produce after initially being sprayed and after storage. The objectives of my thesis are to investigate the reduction of foodborne pathogen after the application of organic acids (malic and lactic acid) and grape seed extract by the electrostatic sprayer on cantaloupe cubes and tomatoes while considering the changes in texture and color over a 12 day storage period at 4ºC.

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Chapter 3: Electrostatic Spray of Food-grade Organic Acids and Plant Extract to Reduce *Escherichia coli* O157:H7 on Fresh-cut Cantaloupe Cubes

Abstract

The objective of the study was to determine the effects of organic acids and plant extract alone and in combination when applied by electrostatic spraying on *E. coli* O157:H7 inoculated cantaloupe cubes. Inoculated cubes were sprayed with malic (M) and lactic (L) acids and grape seed extract (GSE) at different concentrations (alone and various combinations) then stored at 4 °C. Under optimized concentrations, ML 2% was able to reduce *E. coli* O157:H7 by 1.9 logs after 12 days of storage and was significantly different from the controls ($P < 0.05$). Increasing the concentration of M (4%) alone and in combination with L (2%) caused an increase in log reduction (4.6 logs). These treatments can be used as alternatives to commercial sanitizers in order to improve the safety of fresh-cut cantaloupe cubes. Multiple hurdle technology of electrostatic sprayer combined with natural antimicrobials can be effective in improving the safety of food.

Introduction

Outbreaks of foodborne illness have increased over the past decade. Food contamination has caused an estimated 48 million illness and 3000 death per year and lead cause of foodborne illness has been *Salmonella* and *E. coli* O157:H7 (CDC, 2011c). Recently, many of the foodborne illnesses have been linked to fresh or fresh-cut produce like spinach, lettuces, cantaloupe, etc. (CDC 2006; CDC 2010). Outbreaks of *Salmonella spp.*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 have been linked to consumption of cantaloupe (Ukuku *et al.* 2001; CDC 2011a; CDC 2011b). In 1993, cantaloupes were linked to an outbreak of *E. coli* O157:H7 causing 34 people to become sick, but no deaths were reported (Sivapalasingam 2004).

There are strategies to prevent foodborne pathogen contamination on produce. During processing, cantaloupes are washed with water to remove soil from the rind of the melon and disinfectant at appropriate concentrations to reduce foodborne contamination (FDA 2009). Studies have shown that using chlorine as preventative method can be inconsistent (Rodgers *et al.* 2004). Chlorine solution has been demonstrated to reduce *E. coli* and other pathogens by 2 or more logs on lettuce and tomatoes (Akbas and Ölmez 2007; Iturriaga and Escartín 2010) However, other studies have reported less log reduction when chlorine was used at appropriate levels (200 ppm) than alternative antimicrobials like hydrogen peroxide and organic acids (Gil *et al.* 2009; Huang and Chen 2011). Along with unpredictable effectiveness, chlorine can also react with organic matter causing formation of harmful byproducts like trihalomethanes that have been linked to miscarriages and cancer (Huang and Batterman 2009).

Natural preservatives like organic acids and plant extracts have shown the ability to reduce foodborne pathogens on produce and meat products. Organic acids and plant extract are good potential antimicrobials because they have GRAS (generally recognized as safe) status and can be obtained at low cost (Ganesh *et al*. 2010). Malic acid, lactic acid, and grape seed extract are preservatives and have been reported to reduce *E. coli* O157:H7 and *Salmonella* on produce (Raybaudi-Massilia *et al* 2008; Choi *et al* 2012). Different concentrations of lactic acid were applied to pork inoculated with *E. coli*, *Salmonella* Typhimurium, and *Listeria monocytogenes* showed a reduction of 0.79 to 1.18 log CFU/cm^2 (Choi *et al.* 2009). Malic and lactic acids demostrated the ablility to reduce *E. coli* O157:H7 by 1.55 log and 1.74 log when applied to lettuce (Sagong *et al.* 2011). *Listeria monocytogen*es was reduced by ~2 logs on tomatoes that were exposed to grape seed extract (Bisha *et al*. 2010).

This study determines the effects of organic acids (malic and lactic acid) and plant extract (grape seed extract) electrostatically sprayed on *Escherichia coli* O157:H7 inoculated cantaloupe cubes. Electrostatic spraying has been used for pesticide and fungicide application on crops and production lines (Law and Cooper 2001). This technique utilizes high speed air for the formation of small particles (30-60 microns) of solutions. Along with smaller particles, an electrical charge is applied to spray particles (Electrostatic Sprayer System 2011). The charge causes attraction between the droplets and target surface which increase deposition on the target (Law 2001; Electrostatic Sprayer System 2011).

Materials and Methods

Bacterial inoculum preparation

A stock culture of *E. coli* O157:H7 (C7929, Apple cider isolate; Food Microbiology Research Laboratory, Department of Food Science, University of Arkansas) previously stored at -70°C, were used to inoculate in 10 mL of BHI (Brain Heart Infusion, EMD Chemical, Gibbstown, NJ, USA) broth and incubated at 37°C for 24 hr for growth (1st day cultures). The 2nd day culture was prepared by inoculating 10 mL of BHI using 10 µL of 1st day cultures.

BHI:culture mix was then incubated at 37°C for 24 hr. The 2nd day culture was used to inoculate the cantaloupe cubes (10^8 CFU/mL).

Inoculation of Cantaloupe

Fresh cantaloupe melons (*Cucumis melo* var. *cantalupensis*) were purchased from a local grocery store on the day of inoculation. The whole melons were washed with 6.0% sodium hypochlorite solution (Clorox bleach) diluted in deionized water (6.0 mL/L of water), the rinds and seeds removed, and the flesh was cut into cubes (~ 6 g) with a sterile knife. The inoculum was prepared by adding the 2nd day culture (5 mL) to 2 L of sterile deionized water (10^7 CFU/mL). Canteloupe cubes were submerged in the inoculum for 1 min. The samples were placed on trays to dry for 45 min under biosafety hood. The cubes were stored at 4 °C overnight to promote attachment.

Preparation of antimicrobials solutions

Organic acids (malic and lactic acid) and plant extract (grape seed extract) were tested on *E. coli* inoculated cantaloupe cubes to determine antimicrobial effectiveness. Commercial grape seed extract (G) powder was purchased from Mega Natural® Inc. (Madera, CA, USA); malic (M) and lactic (L) acid were purchased from JT Baker (Phillipsburg, NJ, USA) and Fisher Scientific Company (Fair Lawn, NJ, USA). All antimicrobial solutions were prepared the day before spraying respectively. Solutions were prepared using 200 g of DI (deionized water).The different combinations of malic acid, lactic acid, and grape seed extract were prepared based on RSM (response surface method) profile as shown on Table 1 and 2 (JMP [John Macintosh Product] 9.0 software, 2010, SAS Institute, Cary, NC, USA). This method is used to optimize response variable (log reduction) and explore the relationship between variables of the test solutions (M, L, GSE and combinations at different concentrations).

To prepare control test solutions, 200 g of DI (deionized) water was weighed and the pH was adjusted to 2.0 or 1.9 with 1N hydrochloric acid (HCl), which is similar to the test solutions' pH to eliminate the pH factor. The solutions were vacuum filtered through Whatman® No. 4 filter paper (185mm, Schleicher and Schuell) to remove particles that may interfere with spraying.

Electrostatic spray of antimicrobials

Each antimicrobial solution and the control (Table 1 and 2) were sprayed using the electrostatic sprayer (ESS Electrostatic Sprayer XT-3, Electrostatic Spraying Systems Inc., Watkinsville, GA, US) onto inoculated cantaloupe cubes placed on a tray inside spraying chamber. Solutions were sprayed twice for 5 seconds, rotated to spray the side that was touching the tray, and allowed to dry under biosafety hood for 15 min to 30 min. Sprayed cantaloupe cubes were placed individually in sample bags (4 oz., 7.5x18.5 cm, VWR, Sugarland, TX, US) and then stored at 4 °C for up to 12 days to determine efficacy of antimicrobials.

Bacterial enumeration on days 0, 1, 3, 7, 12

Each sample (one cantaloupe cube) was weighed and phosphate buffer saline (PBS, 20 mM, pH 7.0, 8.5 g NaCl, 2.84 g Na_2HPO_4, 2.4 g NaH_2PO_4 in 1 L of deionized water) was added at a volume of twice the weight of the sample to the each bag. The cubes were then stomached for 120 seconds at 8.0 strokes/sec. Stomached samples were serial diluted using PBS and spread-plated on SMAC-CT media (Sorbital MacConkey agar with Cefixime Potassium Tellurite supplement; EMD Chemicals, Gibbstown, NJ, USA) for *E. coli* O157:H7 enumeration. The plates were incubated at 37 °C for 48 hrs. Colony counts were taken after incubation period to enumerate the amount of *E. coli* O157:H7 on the cantaloupe cubes. The procedures were used on the 0, 1st, 3rd, 7th, and 12th day of storage.

Statistical analysis

The experiments were performed with quadruplets. Analysis of variance (ANOVA) was performed using JMP 9.0 (John Macintosh Product 9.0 software, 2010, SAS Institute, Cary, NC, USA). Significant difference was determined at $P < 0.05$.

Results and Discussion

Figures 1and 2 shows the log number of *Escherichia coli* O157:H7 (*E. coli*) remaining on cantaloupe cubes after being sprayed with organic acids and grape seed extract combination and concentrations from Table 1. Cantaloupe cubes treated with pH adjusted water (DI) were not significantly different ($P > 0.05$) from non-treated cantaloupe cubes. DI (pH 2.0) was used as one of the controls to eliminate pH effects as a factor that reduced pathogen on the cube. Organic acids and plant extract were tested alone and in different combinations to determine antimicrobial activity. On the initial day of spraying, each antimicrobial treatment was able to reduce pathogen load. Malic acid (M) and lactic acid (L) at concentration of 2% were able to demonstrate reduction of *E. coli* by 1.0 and 0.6 log CFU/g after 3 days of storage. Grape seed extract (G 2%) illustrated maximum reduction of pathogen load by 0.7 log CFU/g on day 7 of storage period. However, the natural antimicrobials alone were statistically insignificant when compared to each other and to the controls (NT and DI) ($P > 0.05$). Combination of organic acids and plant extract exhibited more reduction of microbial load inoculated on cantaloupe cubes. Malic and lactic acid in combination at concentration of 2% (ML 2%) displayed further reduction as the storage period progressed. By the 12th day of storage, ML 2% reduced *E. coli* on cantaloupe cubes by ~2.0 logs which were significantly difference from NT and DI ($P > 0.05$).

Enumeration results were used to evaluate the effect of malic acid, lactic acid, and grape seed extract on *E. coli* and optimize the concentration for minimizing the log number using JMP prediction profiler (Figure 3). The profiler showed that grape seed extract was not a contributing factor when combined with malic and lactic acid. Grape seed extract has shown to be inferior to organic acids by Ganesh *et al.* (2012). Organic matter found on cantaloupe cubes can possibly react with the grape seed extract and reduce antimicrobial activity (Russell 1999). The figure also illustrated that increasing concentration of organic acids enhanced pathogen reduction. However, as the storage day increased, the antimicrobial activity did not increase.

Concentrations of the organic acids were increased up to 4% to investigate if higher concentration can further reduce *E. coli* (Table 2) over storage period. Reduction was observed with increased concentration of M and L (Figures 4 and 5) over 12 day storage period. M (4%) alone was able to decrease *E. coli* on cantaloupe cubes by 0.8 logs immediately after spraying (Day 0). By the 12th day of storage, a 4.6 log reduction was seen. Decline of pathogen was statistically different from the controls ($P < 0.05$). Different combinations of malic and lactic acid at higher concentration also increased reduction of the pathogen load over 12 day storage period. On the initial day of spraying, a log reduction of 1.3 was observed when cantaloupes were treated with M 4% + L 2%. *E. coli* also decreased by 4.6 logs after 12 day of storage at 4 °C when treated with organic acid combination. M (4%) and M 4% + L 2% were insignificantly different statistically ($P > 0.05$) when compared to each other. The prediction profiler (Figure 6) showed that after 2.0% lactic acid was not as effective over 12 day storage period. Malic acid continued to reduce *E. coli* as the concentration was increase to 4.0% and as the storage period progressed.

Efficacies of M 4% alone and combined with L 2% were either superior or comparable to other sanitizers used on whole melons. Studies have concentrated on applying antimicrobials on the whole cantaloupe to determine the efficacy of pathogen reduction. Mahmoud et al. (2008)

demonstrated significant log reduction of *E. coli* (4.6 log CFU/g) when they treated inoculated whole cantaloupes with chlorine gas (5.0 mg/1). In another study, lactic acid (2%) was able to reduce pathogen load of *E. coli* by 3 logs when whole cantaloupe was stored at 4 °C for 7 days (Alvarado-Casillas *et al.* 2007). Materon (2003) found that sanitizing whole cantaloupe inoculated with *E. coli* by combining lactic acid (1.5%) with or without Tergitol™ (0.3%) was superior to chlorinated wash with or without Tergitol™.

Testing antimicrobials on the flesh helps prevent foodborne illness due to the fact it is the flesh that is consumed, and flesh can become contaminated if the rinds in not properly sanitized. Ukuku *et al*. (2005) found that cantaloupes treated with HPLNC (mixture of hydrogen peroxide, nisin, sodium lactate, and citric acid) demonstrated negative transfer of *E. coli* from surface to flesh on day 0, however there was positive transfer after 7 days of storage with and without enrichment (further incubation of flesh in broth media which was then plated).This is the first time cantaloupe cubes have been inoculated with foodborne pathogen and treated with organic acids and plant extracts by electrostatic spraying. However, one study used bacteriophages (ECP-100) to reduce *E. coli* O157:H7 on the fresh-cut cantaloupe cubes; however the maximum reduction (2.87 log CFU/mL) was observed on day 2 of storage (Sharma *et al.* 2009). Ukuku and Fett (2004) demonstrated that dipping *Salmonella* inoculated cantaloupe cubes in nisin-NaL-KS was able to decrease microbial load by 1.4 log CFU/g.

Organic acids have antimicrobial effects due to the ability to penetrate the cell membrane and to decrease intracellular pH. Molecular size can have an effect on the efficiency of the organic acid (malic acid – 134.09 g/mol and lactic acid – 90.08 g/mol) (Ganesh et al. 2010). Undissociated molecules of malic and lactic acid can enter the bacterial cell more easily to change the internal pH because of their smaller size (Eswaranandam *et al.* 2004). A significant reduction (4.6 log CFU/g) was demonstrated with malic acid at concentration of 4% in combination with lactic acid at 2%. There were no color changes seen when treatments were applied to cantaloupe cubes. Changes in sensory attributes, color, and texture will not be expected to make drastic changes from untreated cubes because small amount of organic acid treatments were applied. Eswaranandam *et al.* (2006) found that malic and lactic acids were incorporated in soy protein coating on cubes did not affect sensory and quality attributes. These natural antimicrobials in combination can be used as alternatives to chlorine or any other sanitizers applied to reduce foodborne contamination on cantaloupe cubes.

Many techniques (dipping and spraying) are used to apply antimicrobials onto fresh produce. Usage of electrostatic sprayer has some advantages over conventional spraying methods. Small charged droplets from the electrostatic sprayer can attach to nonuniform and hidden surfaces (Nam *et al*. 2011). These droplets can move against gravity to cover most of the target surface. Conventional sprayers have poor coverage and sprays large droplets (200 – 500 µm) that can lead to runoff (Kim and Hung 2007). Giles and Blewett (1991) found that electrostatic spray particles increased captan deposit on strawberries when compared to conventional spraying. Less treatment solution was used to control postharvest disease on bananas when using electrostatic sprayer (Law and Cooper 2001). Ganesh *et al.* (2010) used electrostatic spray to apply organic acids and plant extracts as a method of reducing *Salmonella* Typhimurium on spinach and found the application techniques was more effective than conventional spraying.

Malic acid (4%) alone and in combination with lactic acid (2%) was able to reduce *E. coli* O157:H7 on fresh cut cantaloupe cubes when sprayed with the electrostatic sprayer. Antimicrobial activity of natural preservatives increased over 12 day storage period. Applying the organic acid with the electrostatic sprayer is multiple hurdle technology that can be used to improve foodborne safety of fresh-cut cantaloupe cubes.

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TABLE 1.
RESPONSE SURFACE METHOD DESIGN OF VARIOUS TREATMENTS WITH VARYING CONCENTRATIONS AND COMBINATIONS OF MALIC AND LACTIC ACID AND GRAPE SEED EXTRACT SPRAYED WITH ELECTROSTATIC SPRAYER

	Treatments		
Number of treatments	M (%)	L (%)	G (%)
1	2	0	0
2	0	2	0
3	0	0	2
4	1	1	0
5	2	2	0
6	1	0	1
7	2	0	2
8	0	1	1
9	0	2	2
10	1	1	1
11	2	2	2
12	2	1	1
13	1	2	1
14	1	1	2
15	DI water (pH 2.0)		
16	NT		

DI (deionized water), NT (no treatment), M (malic acid), L (lactic acid), G (grape seed extract)

TABLE 2.
RESPONSE SURFACE METHOD DESIGN OF VARIOUS TREATMENTS WITH VARYING INCREASED CONCENTRATION AND COMBINATIONS OF MALIC AND LACTIC ACID SPRAYED WITH ELECTROSTATIC SPRAYER

	Treatments	
Number of treatments	M (%)	L (%)
1	4	0
2	0	4
3	2	2
4	3	3
5	4	4
6	4	2
7	4	3
8	2	3
9	2	4
10	3	2
11	3	4
12	DI water (pH 1.9)	
13	NT	

DI (deionized water), NT (no treatment), M (malic acid), L (lactic acid)

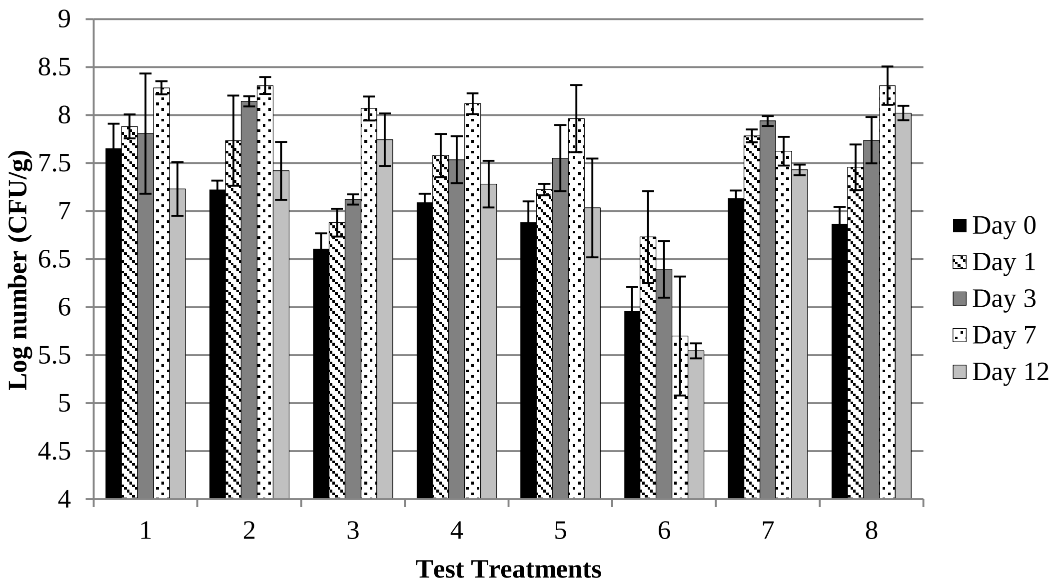

FIGURE 1: EFFECTS OF MALIC AND LACTIC ACIDS AND GRAPE SEED EXTRACT ELECTROSTATICALLY SPRAYED ON CANTALOUPE CUBES INOCULATED WITH *E. COLI O157:H7* OVER 12 DAYS.

Values are means log numbers of quadruplet analysis and error bars indicate the standard error of means. $P < 0.05$. Test treatments: 1-NT (no treatment), 2- DI (pH adjusted 2.0 deionized water), 3-2% M (malic acid), 4- 2%L (lactic acid), 5- 1%M + 1%L, 6-2%M + 2%L, 7-2%G (grape seed extract), 8- 1%M + 1%G.

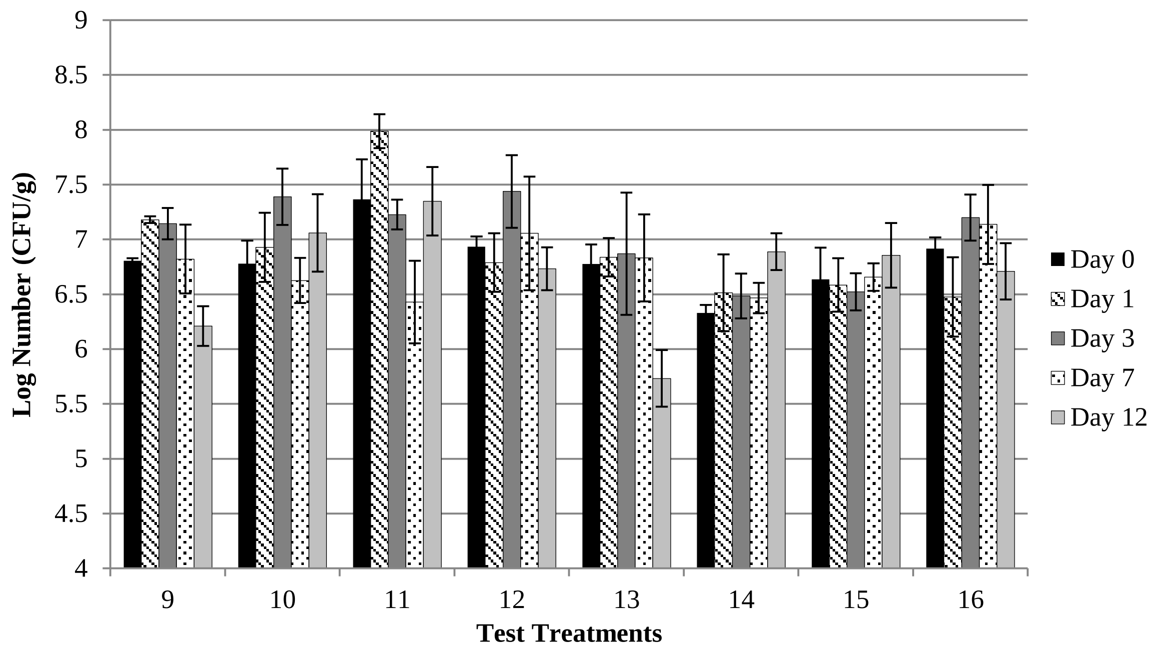

FIGURE 2: EFFECTS OF MALIC AND LACTIC ACIDS AND GRAPE SEED EXTRACT ELECTROSTATICALLY SPRAYED ON CANTALOUPE CUBES INOCULATED WITH *E. COLI O157:H7* OVER 12 DAYS.

Values are means log numbers of quadruplet analysis and error bars indicate the standard error of means. $P < 0.05$. Test treatments: 9- 2% M + 2%G, 10-1%L + 1%G, 11-2%L + 2%G, 12- 1%M + 1%L + 1%G, 13-2%M + 2%L + 2%G, 14-2%M + 1%L + 1%G, 15-1%M + 2%L + 1%G, 16-1%M + 1%L + 2%G.

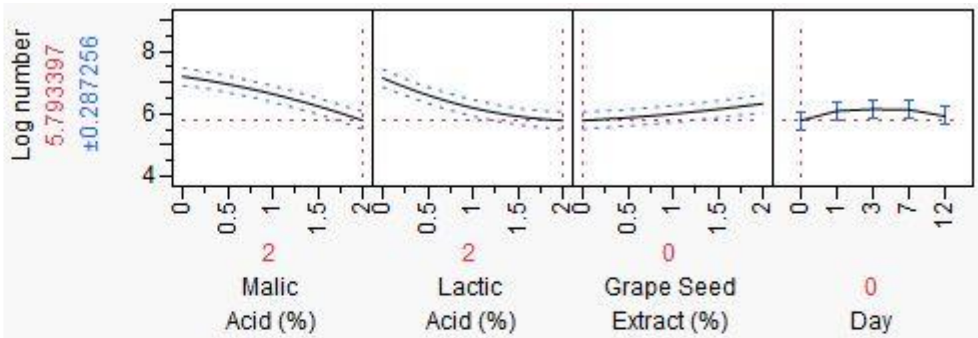

FIGURE 3: PREDICTION PROFILER WITH OPTIMIZED CONCENTRATION OF ORGANIC ACIDS AND PLANT EXTRACT FOR LOWEST LOG NUMBER OF *ESCHERICHIA COLI* O157:H7 OVER 12 DAY OF STORAGE AT 4 °C.

Point where the dotted vertical and horizontal lines meet indicates the concentration of M, L, and G that contributed to highest antimicrobial activity.

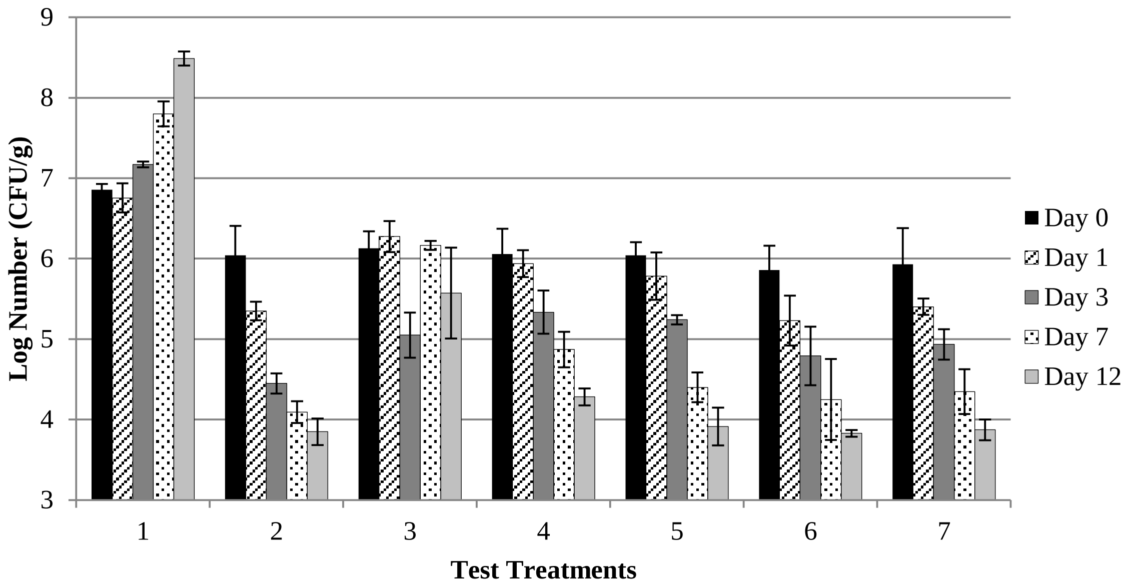

FIGURE 4: EFFECTS OF MALIC AND LACTIC ACIDS ELECTROSTATICALLY SPRAYED ON CANTALOUPE CUBES INOCULATED WITH *E. COLI O157:H7* OVER 12 DAYS.

Values are means log numbers of quadruplet analysis and error bars indicate the standard error of means. $P < 0.05$. Test treatments: 1-DI (pH adjusted 1.9 deionized water), 2-4%M (malic acid), 3-4%L (lactic acid), 4-2%M + 2%L, 5-3%M + L3%, 6-4%M + 4%L, 7-3%M + 4%L.

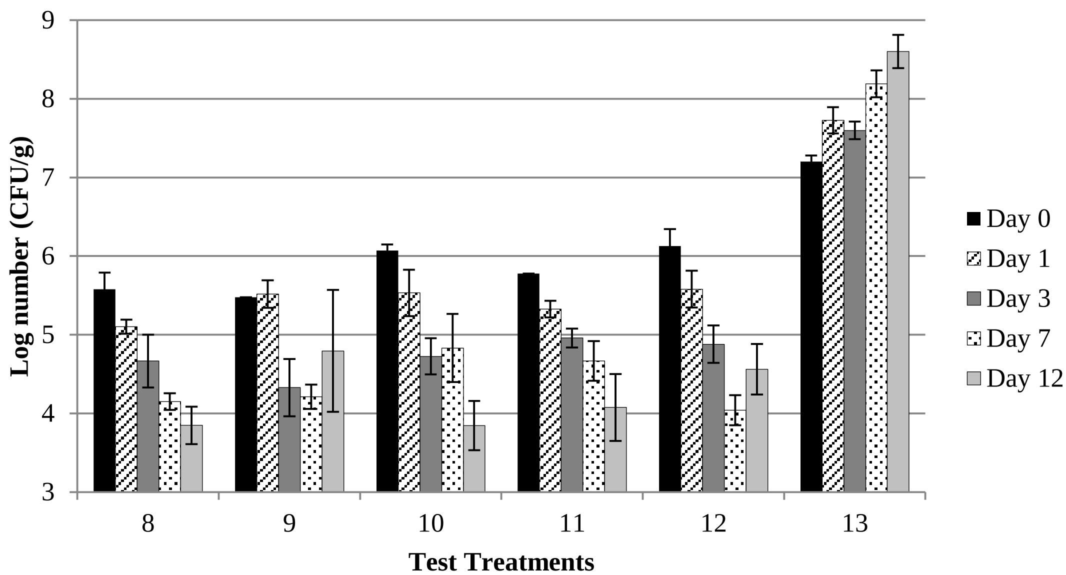

FIGURE 5: EFFECTS OF MALIC AND LACTIC ACIDS ELECTROSTATICALLY SPRAYED ON CANTALOUPE CUBES INOCULATED WITH *E. COLI O157:H7* OVER 12 DAYS.

Values are means log numbers of quadruplet analysis and error bars indicate the standard error of means. $P < 0.05$. Test treatments: 8-4%M + 2%L, 9-4%M + 3%L, 10-2%M + 3%L, 11-2%M + 4%L, 12-3%M + 2%L, 13- No treatment (NT).

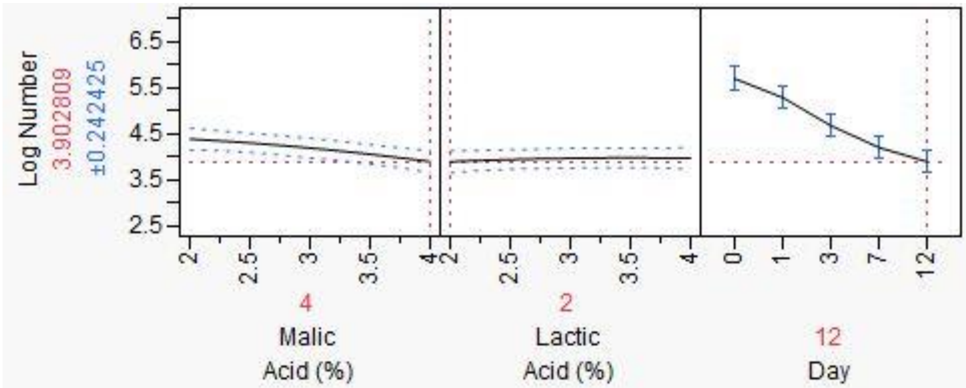

FIGURE 6: PREDICTION PROFILER WITH OPTIMIZED CONCENTRATION OF ORGANIC ACIDS FOR LOWEST LOG NUMBER OF *ESCHERICHIA COLI* O157:H7 OVER 12 DAY OF STORAGE AT 4 °C.

Point where the dotted vertical and horizontal lines meet indicates the concentration of M and L that contributed to highest antimicrobial activity

Appendix

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August 12, 2013

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TO WHOM IT MAY CONCERN:

I hereby state that Leighanna M. Massey is the first author of "Electrostatic Spray of Food-grade Organic Acids and Plant Extract to Reduce *Escherichia coli* O157:H7 on Fresh-cut Cantaloupe Cubes" and she completed more than 51% of the work of the manuscript.

Sincerely,

Dr. Navam S. Hettiarachchy
Major Advisor/ Thesis Director

Chapter 4: The effects of electrostatic spray of malic acids, lactic acid, and grape seed extract at 0, 1, 2, 3, and 4 % on inoculated surfaces of cantaloupe cubes and tomatoes at 4°C

Abstract

The objective of the study was to determine the effect of organic acids and plant extract on *Salmonella* Typhimurium (ST) and *Escherichia coli* O157:H7 (EC) inoculated fruits and how the electrostatic spraying technique compares to conventional spraying. Cantaloupe cubes and tomatoes were inoculated with ST and EC, then electrostatically sprayed with different concentrations of organic acids and grape seed extract. Sprayed produce was monitored over 11 and days of storage at 4ºC. Malic acid (4%), alone and combined with lactic acid (2%) demonstrated the most reduction of ST (reduced by 3.3 and 3.6 log CFU/g) and EC (4.6 log CFU/g) on cantaloupe cubes during the storage period. Lactic acid alone and in different combinations with malic acid were able to decrease EC (2.3 log CFU/g) and ST ($\geq$ 3.7 log CFU/g) on tomatoes after 12 days of storage. Compared to conventional spraying, electrostatic spraying of organic acids on cantaloupe cubes reduced more EC and ST. However, there was no difference between sprayers when applying natural antimicrobials to tomatoes. Natural antimicrobials can be used to improve the safety of fresh cut cantaloupe cubes and tomatoes. Usage of multiple hurdle technology in the produce can be used along with current practices to enhance the safety of food.

Introduction

The promotion of healthier lifestyles has increased the consumption of fresh fruits and vegetables, organic and non-organic (Berger *et al.* 2010). In 2010, there was an increase in fruit and vegetable consumption by 1 % from 2009. As of 2012, there has been a 3% increase in fresh cut fruits and vegetables consumption despite the increase in retail price (Dean 2012). With this increase, there has been a decrease in consumption of processed vegetables. Along with a healthier lifestyle, consumption of fresh fruits and vegetables has led to an increase of foodborne illnesses (Lucier and Glaser 2011).

Escherichia coli O157:H7 and *Salmonella* have emerged as the important pathogenic contaminates on fresh produce. *Salmonella* is the most common infection as of 2010, and *E. coli* O157:H7 incidence has not significantly changed in the past 10 years (CDC 2011a). These pathogens cause diarrhea, abdominal pain, and mild fever that last for 4 to 7 days. However, many cases of foodborne illnesses do not get reported unless patients go to the hospital (CDC 2011b). Suppliers and industries incorporate GMP (good manufacturing practices), GAP (good agricultural practices), and HACCP (Hazard Analysis Critical Control Points) as primary control of pathogens along with washing the produce by physical and chemical means to help reduce the occurrence of infection (Samara and Koutsoumanis 2009; Parish *et al.* 2003).

Produce is washed to remove soil, plant debris, and microorganism that are found on the surface. Chlorine is the most widely used sanitizing agent to wash produce. However, chlorine is less effective against bacteria attached to or embedded within the surface (Sapers *et al.* 2006), and may have harmful effects to the produce and consumers (Ganesh *et al.* 2010). Because of this, natural antimicrobials have been studied to provide safe alternatives.

Organic acids and plant extracts are promising antimicrobial agents because they are natural, can be obtained at a low cost, and most have GRAS status (Choi *et al.* 2009, Perumalla and Hettiarachchy 2011). Numerous studies have found that organic acids and plant extracts have the ability to reduce *Salmonella* and *E. coli* O157:H7 from the surfaces of various produce (Gutierrez *et al.* 2009, Sagong *et al.* 2011). In addition to antimicrobial properties, plant extracts have other health benefits that they can contribute to produce like antioxidants and anticarcinogens (Perumalla and Hettiarachchy 2011).

Organic acids and plant extracts have been applied in different forms to food products because of their antimicrobial properties. Edible films containing malic acids, grape seed extract, and nisin applied to produce and meat products have demonstrated the ability to reduce *Salmonella*, *Listeria monocytogenes*, and *E. coli* O157:H7 (Eswaranandam *et al.* 2004; Gadang *et al.* 2008). The use of natural antimicrobial along with a novel technology has the potential to be used as alternative methods to reduce the occurrence of foodborne illness.

The objective of this investigation is to study the effects of electrostatic spray of malic acid, lactic acid, and grape seed extract at different concentrations and combinations on cantaloupe cubes and tomatoes inoculated with *Salmonella* Typhimurium and *E. coli* O157:H7.

Materials and Methods

Bacterial inoculum preparation

Minus 70°C frozen stock culture of *Salmonella* Typhimurium and *Escherichia coli*O157:H7 were used to inoculate in 10 mL of BHI (Brain Heart Infusion, EMD Chemical Inc., Gibbstown, NJ, US) broth and incubated at 37°C for 24hr. The 2nd day cultures were prepared by inoculating another 10 mL of BHI using 10 µL of 1st day culture. Cultures were incubated at 37°C for 24 hr.

Inoculation of Produce

Fresh cantaloupes (*Cucumis melo* var. *cantalupensis*) and tomatoes (*Solanum lycopersicum* var. *cerasiforme*) were purchased from a local grocery store on the day of inoculation. Each sample was washed in sodium hypochlorite solution (6 mL of Clorox containing 0.6% NaOCl added to 1 L of deionized (DI) water) and rinsed in DI water. Cantaloupes' rinds were removed from the flesh and were cut into approximately 6 g cubes with a sterile knife. Inoculum was prepared by adding 5mL of 2nd day culture to 2 L of sterile DI water (10^9 - 10^7cfu/mL). The samples were then submerged in the inoculum for 1 - 2 minute and placed on porous tray to dry for 40 min under biosafety hood. The samples were placed in plastic bags and stored at 4°C overnight to promote attachment.

Whole tomatoes, after stem removal, were washed with sodium hypochlorite solution (similar cantaloupe wash), and left to dry for 15 min. For inoculation, 10 spots of 10 µL of inoculum (total of 100 µL) were placed round the tomatoes to facilitate drying and to prevent runoff. Inoculated tomatoes were left under biosafety hood at 22°C overnight to promote microbial attachment.

Preparation of antimicrobial solutions

All solutions were prepared the day before spraying. Commercial grape seed extract powder was purchased from Mega Natural® Inc. (Madera, CA, USA). Malic and lactic acid were purchased from JT Baker (Phillipsburg, NJ, USA) and Fisher Scientific Company (Fair Lawn, NJ, USA). The different concentrations and combinations of malic and lactic acid and grape seed extract were prepared based on RSM (response surface method) profile. The test solutions were prepared using 200 g of DI water. The control test solutions were prepared using DI water (200g) with an adjusted pH levels to 2.0 and 1.9 (using 0.1N HCl) which is similar pH to the other test solutions to eliminate the pH factor. The solutions were then vacuum-filtered through Whatman® No. 4 filter paper to remove impurities that may interfere with sprayer. RSM was performed for each produce to obtain optimized concentrations for both *E. coli* O157:H7 and *Salmonella* Typhimurium.

Spraying of antimicrobials

The electrostatic sprayer was used for this study (Electrostatic Spraying Systems Inc., Watkinsville, GA, US). The different test solutions including the control (DI water) were sprayed using the electrostatic sprayer onto inoculated cantaloupe cubes and tomatoes placed on a tray inside closed chamber with opening for sprayer nozzle. Garden hand sprayer (Professional Plant and Garden Sprayer [32 oz.], The Bottle Crew, W. Bloomsfield, Michigan) was used to simulated conventional sprayer. Solutions were sprayed in equal amounts using the different sprayers and allowed to dry for 30 min. Sprayed samples were then bagged individually (4 oz., 3 x 7 in, VWR, Sugarland, TX, US) and stored at 4°C.

Enumeration of bacteria during storage for day 0, 1, 3, 7, 11, 12

Each sample (1 cantaloupe cube and 1 tomato) was weighed and phosphate buffer saline (PBS, 20 mM, pH 7.0, 8.5 g NaCl, 2.84 g Na_2HPO_4, 2.4 g NaH_2PO_4 in 1 L of DI water) added at a volume of twice the weight of the sample to the sample bags. The samples were stomached for Foon XLT-4 (Xylose Lysine Tergitol 4) agar (Hi-Media Laboratories Pvt. Ltd., India) and SMAC (Sorbitol MacConkey) agar with CT (Cefixime Potassium Tellurite) supplements (EMD Chemicals, Gibbstown, NJ, USA) for *Salmonella* Typhimurium and *E. coli* O157:H7 enumeration. The plates were incubated at 37°C for 48 hrs. Colony counts were taken after 48 hr incubation period. The procedures were followed on the 1st, 3rd, 7th, 11th and 12th day of storage.

For cantaloupe, quadruplet samples were taken and eight samples were taken of tomato on each observation day.

Statistical Analysis

Results were analyzed with JMP (John's Macintosh Product) 10.0 software (SAS Inst. Inc., Cary, N.C., USA) using ANOVA (analysis of variance) and significant differences between results will be estimated with $P<0.05$.

Results and Discussion

Antimicrobial Effects on Cantaloupe

Over the years, fresh cut produce has performed best under the value added category. Increase in sales of cantaloupe cubes is based on need for single service products because families are smaller and it is convenient for consumers (Dean 2012). As stated previously, with the increase in sales and consumption of cantaloupe cubes, there is a need for alternative methods to improve the safety of the product. Most researchers have performed studies on how alternative antimicrobials and/or technologies can decrease bacterial loads on the outer layer of the cantaloupe (Materon 2003; Palekar *et al.* 2004; Alvarado-Casillas *et al.* 2007; Mahmoud 2012). It is important that alternative antimicrobials and application techniques are found for usage on cantaloupe cubes. These alternative techniques are important because after sanitizing the outer surface of the cantaloupe transference of pathogens from the netted matrix to the edible flesh has been observed (Ukuku *et al.* 2005).

In this study, prepared antimicrobials were electrostatically sprayed on cantaloupe cubes that had been inoculated with *Salmonella* Typhimurium (ST) (Figure 1). Cubes treated with control solution (pH adjusted DI water) showed a maximum reduction of 0.3 log of ST. When compared to cubes that were untreated, there was no significant reduction of the pathogens (P >

0.05). Natural antimicrobials were then applied to inoculated samples alone and combination at various concentrations (Table 1). Natural antimicrobials were reduced ST (0.2 – 0.5 log CFU/g) on cantaloupe cubes; however the reduction was not significantly different than the controls. Combination of organic acids and grape seed extract demonstrated further decreases in foodborne pathogen inoculated on samples. ML 2.0 %, MLG 2.0 %, L2 + MG, and G2 + ML were able to reduce *Salmonella* by approximately1.0 log CFU/g by the 11th day of storage.

The results were analyzed using a JMP prediction profiler. This feature is used to aid in determining the effect of organic acids and grape seed extracts on foodborne pathogens and optimizing the concentration for minimizing the log number remaining on the cantaloupe cubes. The profile shows that malic and lactic acid enhanced pathogen reduction as the concentration increased. Grape seed extract was not much of a factor in removing ST from the cantaloupe cubes. Reduced antimicrobials activity can be due to an excess of organic matter on the produce or in the solution (Ganesh *et al*. 2010)

Increase concentrations (up to 4.0 %) of malic and lactic acids were used to investigate if microbial reduction would be enhanced (Table 2). As seen previously, cubes treated with pH adjusted solution (control) reduced the pathogens, but the microbial populations remaining were not significantly different ($P > 0.05$) from cubes left untreated. On the initial day of spraying, M 4.0 %, M 4.0 % + L 2.0 %, M 4.0 % + L 3.0 %, and ML 4.0 % were able to reduce ST by 1.0 – 1.5 logs, which was significantly different from the controls (Figure 2). Similar log reductions were seen by Ukuku and Fett (2004) after 7 days of storage at 5°C when applying nisin-NaL-KS on cubes from *Salmonella inoculated* cantaloupes. Maximum log reduction of ST was seen at the end of the storage period (3.3 log CFU/g with malic acid (4.0%) alone and 3.6 log CFU/g when combined with L 2.0 %).

The organic acid exhibited the ability to reduce more *E. coli* (previously seen in Chapter 3) than *Salmonella* on the cantaloupe cubes. This can be due to the sensitivity of the *E. coli* strain used. Other studies have shown that some *E. coli* strains are more sensitive than *Salmonella* strains. Yossa et al. (2012) found greater *E. coli* reduction (3.4 log CFU/g) on spinach leaves than *Salmonella (*2.4 log CFU/g) after being treated with Sporan (blend of clove, rosemary, and thyme oil) plus acetic acid. Another study showed EO water decreased *E. coli* by < l.0 log CFU/mL and *Salmonella* Enteritidis and *Listeria monocytogenes* by 1.0 and 1.3 log CFU/mL after 5 minutes of exposure at 4°C (Venkitanarayanan *et al.* 1999).

Cantaloupe: Conventional vs. Electrostatic Spray

The electrostatic sprayer has many advantages over using a conventional sprayer. The first advantage is related to the droplet size that is deposited on the spray target. Conventional sprayers deposit large droplets (200 to 500 μm) that can lead to runoff which can reduce the effectiveness of antimicrobial treatments (Giles and Blewett 1991). The electrostatic sprayer emits smaller droplets (30 to 60 μm) with an applied charge (Electrostatic Spraying Systems 2011). This provides enhanced coverage over the target surface and reduces the occurrence of runoff. Also, the electrostatic sprayer uses 50% less of the antimicrobial solution (Kim and Hung 2007).

A comparative study between conventional sprayer and electrostatic sprayer was done to determine which technique displays more log reduction. For EC, on day 0, M 4% demonstrated a significant difference between the sprayers (ES: 1.1 log CFU/g; CS: 0.6 log CFU/g) on reducing EC on cantaloupe cubes (Figure 3). After day 0 of spraying, M 4% alone and combined with L 2% sprayed with electrostatic sprayer reduced more EC than antimicrobials sprayed with conventional sprayer. On the initial day of spraying, the combination of malic acid (4%) and lactic acid (2%) was able to reduce ST levels by 1.2 log CFU/g as was seen previously when sprayed using the electrostatic sprayer. When conventional spraying was used, only 0.2 log reduction was observed. As the storage period increased, conventional application of antimicrobial observed maximum reduction of 0.9 log CFU/g by end of storage period. Progression of ST reduction was seen each day of storage when M 4% was applied by the electrostatic sprayer (0 to 3.0 log CFU/g) (Figure 4). However, when M 4% was applied with the conventional sprayer, less log reduction (0 to 0.7 log CFU/g) was seen, and there was no significant difference between conventionally sprayed cantaloupe cubes and cubes left untreated or sprayed with pH adjusted DI water. Law and Cooper (2001) found that clusters of bananas sprayed with an electrostatic sprayer provided better postharvest crown-rot control while using half the amount of treatment solution when compared to a conventional hydraulic sprayer. Ganesh *et al.* (2010) found that malic acid combined with grape seed extract was more effective in reducing *Salmonella* Typhimurium on spinach when sprayed from electrostatic sprayers than conventional sprayer.

Antimicrobials Effect on Tomatoes

Tomatoes were electrostatically sprayed with organic acids and grape seed extract and the remaining log numbers of ST and EC were enumerated (Figure 5 and 6). Initial amounts placed on the tomatoes were 7 log CFU/mL, but only about 4 -5 log CFU/mL was attached on to the surface of the tomato. Usually, bacterial cells require nutrients and/or surface that are rough and netted to attachment to that facilitate the growth. The surface of tomatoes is smooth which could lead to reduced attachment.

For EC, maximum 1.0 log reduction of tomatoes treated with pH adjusted DI water was seen by day 12 which was not significantly different when compared to tomatoes not treated with antimicrobials. The pH adjusted deionized water was able to reduce ST (0.6 log reduction) by day 3. This log reduction was significantly different ($P < 0.05$) when compared to untreated tomatoes. A factor that can influence antimicrobial activity is the solutions ability to change the pH of the environment (In *et al.* 2013). This demonstrates that the acidic pH of the water or just the washing the tomatoes with water had an effect on *Salmonella* attached to the surface of tomatoes. The natural antimicrobial solutions were compared to the two controls (NT and DI). Alone, lactic (L) and malic (M) acid and grape seed extract (GSE) were only able to lower ST count by 1.0 log CFU/g and 1.3 log CFU/g and were statistically significantly different from the controls by the end of storage period. L and GSE alone demonstrated the least reduction of EC over the 12 storage period, while M demonstrated reduction of 1.2 log CFU/g by day 7. Combinations of malic and lactic acid exhibited increases in log reduction for ST and EC. The highest log reduction of ST was seen on day 3 (2.8 log reduction by ML 2%). Again, microbial load of EC was decreased by 1.2 logs by combination of lactic acid and malic acid (2%).

The data was analyzed using a JMP predicted profiler to determine the effect of organic acids and plant extract on reducing ST or EC and optimizing the concentration to minimize the log number. The profiler demonstrated that 2% concentration of malic and lactic acid was able to reduce foodborne pathogen on tomatoes. However, grape seed extract did not exhibit much antimicrobial effect. This demonstrates that organic acids play a bigger role in decreasing ST and EC on cantaloupe cubes than grape seed extract. Other plant extracts have shown promise as natural antimicrobials. Essential oil from mustard reduced ST more than 5 logs after 18 hours of exposure (Yun *et al.* 2013). A study demonstrated that thymol, thyme oil, and carvacrol as able to reduce *Salmonella* spp. to undetectable levels after 5 and 10 min washes when compared to chlorine treatments (Lu and Wu 2010).

The concentrations of organic acids were increased (4%) using RSM (Table 2) to determine whether microbial load can be reduced further (Figure 7 and 8). The treatments were significantly different from controls as seen previously with lower concentrations of organic acids. Many of the treatments were able to reduce the ST pathogen load from 0.6 – 1.6 log CFU/g immediately after spraying. On the initial day of spraying, L 4% demonstrated the most microbial reduction (1.1 log CFU/g). As the storage increased, pathogen load decreased to undetectable levels by three treatments. Notable treatments, L 4% and M 2% + L 3%, reduced ST to undetectable levels by day 3 and for the following days of observation (≥ 3.7 logs). Similar reduction was observed by Gϋndϋz *et al.* (2009) after immersing ST inoculated tomatoes in antimicrobial containing 1000 ppm of myrtle oil for 5, 10, and 15 minutes. L alone (4%) and combination of M 3% and L 2% and was able to reduce ST to undetermined levels, giving a log reduction of 4.3 and 3.7 log CFU/g after 3 days of storage. Gurtler *et al*. (2012) inoculated stem scars of tomatoes and dipped numerous sanitizers with inorganic and organic acids. The highest log reduction of *Salmonella seen* was 4.61 log CFU when 1.5% phosphoric acid + 1.5% lactic acid applied to the stem scar.

Alone, malic and lactic acid demonstrated maximum reduction of EC load by 1.2 and 1.7 log CFU/g on day 7 of storage period. However, the combination of M 3% + L 2% exhibited a progression of reduction over the 12 day storage period. 2.3 log CFU/g was the maximum log reduction by the end of the storage period. A previous study by Gyawali *et al*. (2011) found that 0.2% lactic acid alone and combined with 40 ppm of copper was able to reduce EC on tomatoes by 2.29 log CFU/tomato. Another study showed that acidic electrolyzed water was able to produce a 5.0 log reduction of *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium after being treated for 5 minutes (Park et al. 2009).

Tomatoes: Conventional vs. Electrostatic Spray

Comparative study was performed to determine if conventional spraying of antimicrobial produced similar results as antimicrobials sprayed using the electrostatic sprayer. Using a sprayer system can reduce cross contamination and amount of solution needed. Chang and Schneider (2011) found that an overhead spray system with rolling brushes using NaOCL reduced *Salmonella* by 5.5 log CFU/mL compared to 3.3 log CFU/mL from simulated flume. Antimicrobials that demonstrated reduction of *Salmonella* were sprayed on to inoculated tomatoes and stored for 12 days. Previously, we found that log number remaining from tomatoes treated with control solutions was significantly different from tomatoes untreated. Sterile deionized water (DI) was used to determine if pH of the control solution was a factor in reducing pathogen load. Control solution and sterile DI water did not exhibit any significant differences between each other during the 12 day storage period when sprayed with each sprayer.

Antimicrobials sprayed with conventional and electrostatic sprayer reduced ST by 2.0 logs by day 3 and 7 (Figure 9). However, when comparing electrostatic spraying to conventional spraying, there was no significant difference in log number remaining of ST was on the tomatoes for any day of storage. Reduction of EC after initially being sprayed with electrostatic sprayer (1.6 log CFU/g) was significantly different from tomatoes sprayed with the conventional sprayer (1.0 log CFU/g) (Figure 10). Microbial load continued to decrease as the storage period increased and there was a difference ($P < 0.05$) in log number remaining after using the different sprayer where their electrostatic sprayer reduced more EC than the conventional sprayer. A study comparing electrostatic spraying to uncharged (conventional) spraying found peracetic acid applied to different food handling and processing surfaces was more effective in reducing *Salmonella* when sprayed using electrostatic sprayer (Lyons *et al.* 2011). Results from the EC study are in agreement with a study where white mold infection was reduced more when fungicide was applied by electrostatic sprayer on beans (Storozynsky 1998).

Conclusion

Natural antimicrobials can be used on cantaloupe cubes and tomatoes to reduce foodborne pathogens. The use of the electrostatic spray as an application method for cantaloupe cubes was better than conventional spraying. Use of the novel technology to reduce *E. coli* O157:H7 on tomatoes is promising. These components (natural antimicrobials solutions and electrostatic sprayer) have the potential to be used to enhance the safety of fresh fruits and vegetables.

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Table 1. Concentrations and Combinations of Antimicrobial Treatments Sprayed by Electrostatic Sprayer Based on Response Surface Method Design

	Treatments		
Number of Treatments	M[a] (%)	L[b] (%)	G[c] (%)
1	2	0	0
2	0	2	0
3	0	0	2
4	1	1	0
5	2	2	0
6	1	0	1
7	2	0	2
8	0	1	1
9	0	2	2
10	1	1	1
11	2	2	2
12	2	1	1
13	1	2	1
14	1	1	2
15		NT[d]	
16		DI[e] water (pH 2.0)	

[a]M (malic acid), [b]L (lactic acid), [c]G (Grape Seed Extract), [d]NT (not treatment), [e]DI (deionized)

Table 2. Increased Concentration of Antimicrobial Treatments Applied with Electrostatic Sprayer Based on Response Surface Method Design

	Treatments	
Number of treatments	M[a] (%)	L[b] (%)
1	4	0
2	0	4
3	2	2
4	3	3
5	4	4
6	4	2
7	4	3
8	2	3
9	2	4
10	3	2
11	3	4
12	DI[c] water (pH 1.9)	
13	NT[d]	

[a]M (malic acid), [b]L (lactic acid), [c]NT (not treatment), [d]DI (deionized)

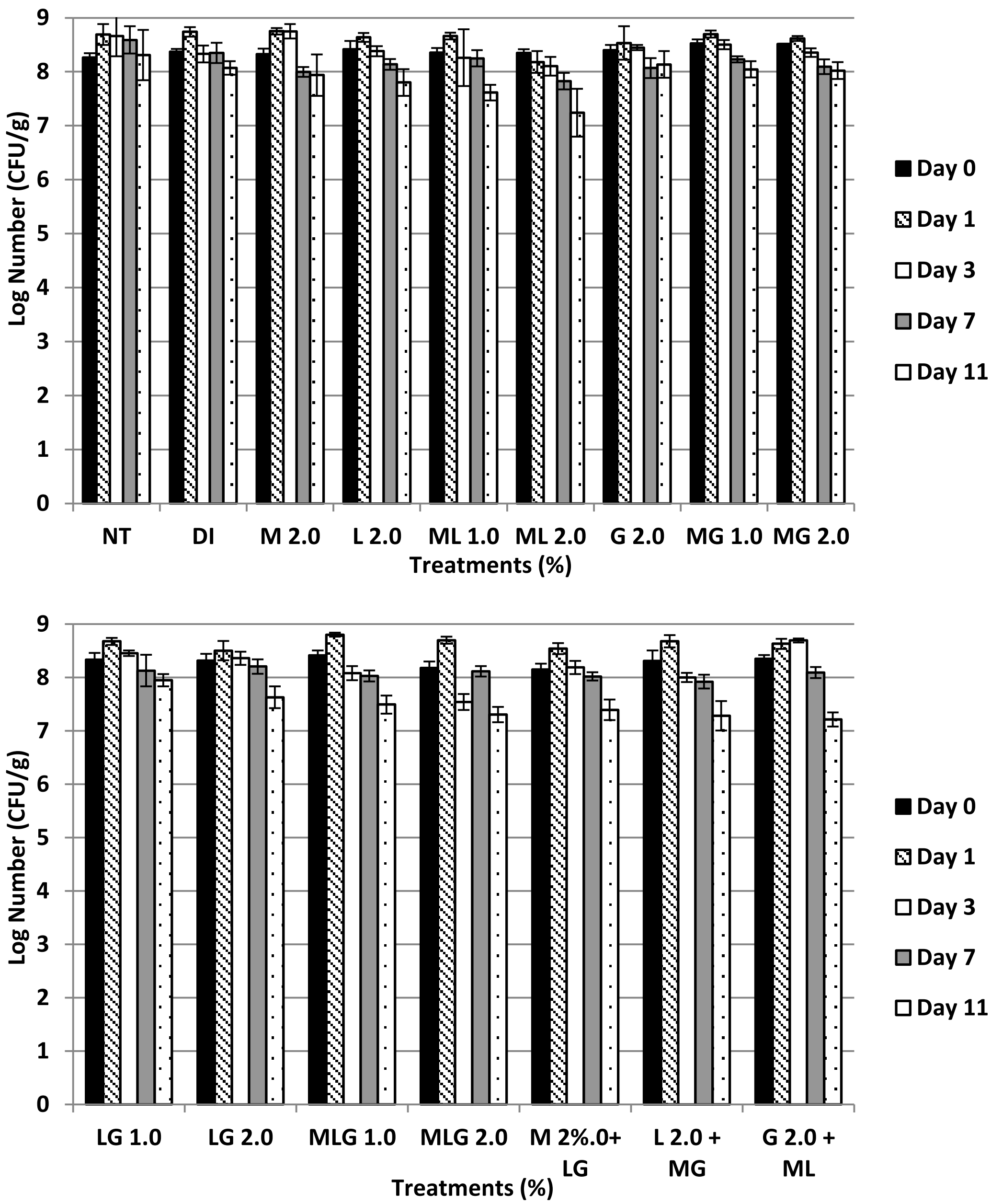


FIGURE 1: EFFECTS OF ORGANIC ACIDS AND GRAPE SEED EXTRACT ELECTROSTATICALLY SPRAYED ON CANTALOUPE CUBES INOCULATED WITH *SALMONELLA* TYPHIMURIUM OVER 11 DAYS.

Values are means log numbers of quadruplet analysis and error bars indicate the standard error of means. $P < 0.05$. Test treatments: NT (untreated), M (malic acid), L (lactic acid), G (grape seed extract), DI (pH adjusted (2.0) deionized water).

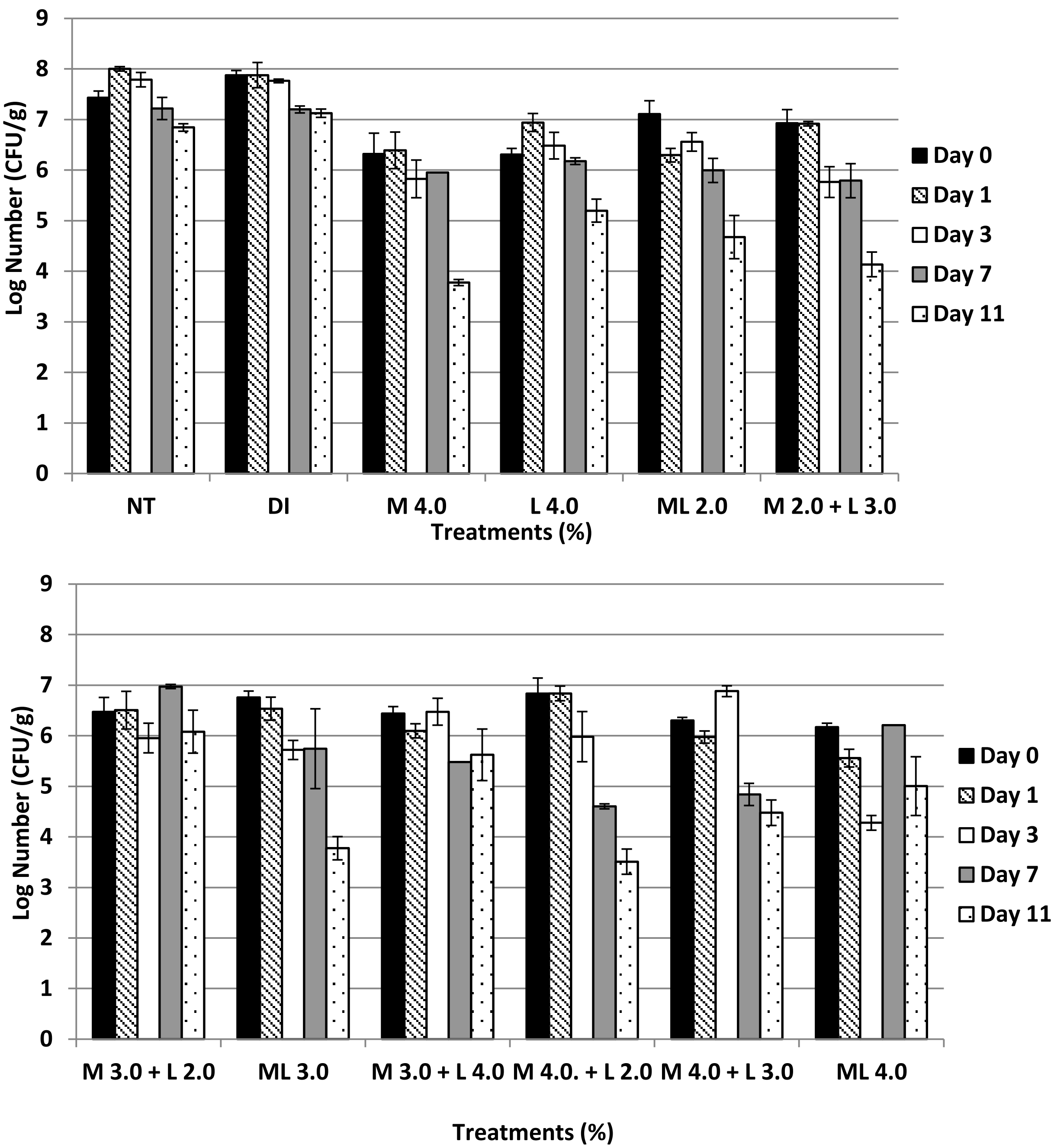


FIGURE 2: EFFECTS OF MALIC AND LACTIC ACIDS ELECTROSTATICALLY SPRAYED ON *SALMONELLA* INOCULATED CANTALOUPE CUBES OVER 11 DAYS. Values are means log numbers of quadruplet analysis and error bars indicate the standard error of means. $P < 0.05$. Test treatments: NT (no treatment), M (malic acid), L (lactic acid), DI (pH adjusted (1.9) deionized water).

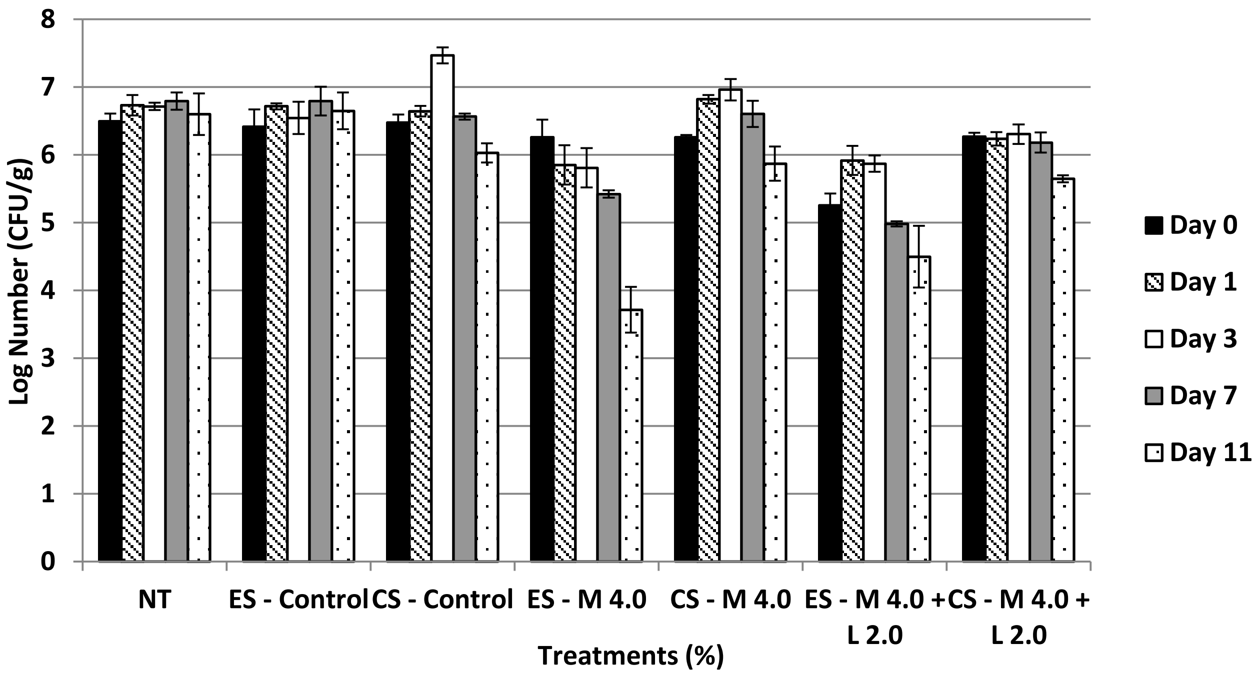


FIGURE 3: LOG REMAINING OF *SALMONELLA* TYPHIMURIUM ON CANTALOUPE CUBES SPRAYED WITH NATURAL ANTIMICROBIALS BY CONVENTIONAL AND ELECTROSTATIC SPRAYER.

Values are means log numbers of quadruplet analysis and error bars indicate the standard error of means. $P < 0.05$. Test treatments: Test treatments: ES (electrostatic sprayer), CS (conventional sprayer), NT (untreated cantaloupe cubes), Control (pH adjusted (1.9) deionized water), M (malic acid), L (lactic acid).

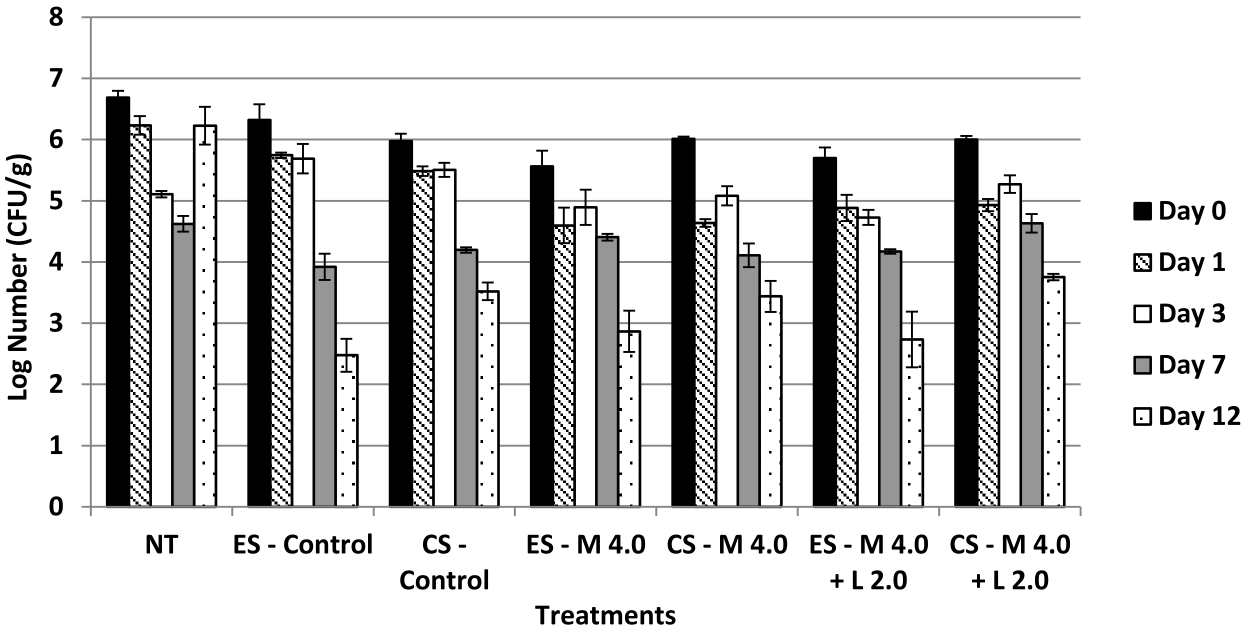


FIGURE 4: LOG REMAINING OF *E. coli* O157:H7 ON CANTALOUPE CUBES SPRAYED WITH NATURAL ANTIMICROBIALS BY CONVENTIONAL AND ELECTROSTATIC SPRAYER.

Values are means log numbers of quadruplet analysis and error bars indicate the standard error of means. $P < 0.05$. Test treatments: ES (electrostatic sprayer), CS (conventional sprayer), NT (untreated cantaloupe cubes), Control (pH adjusted (1.9) deionized water), M (malic acid), L (lactic acid).

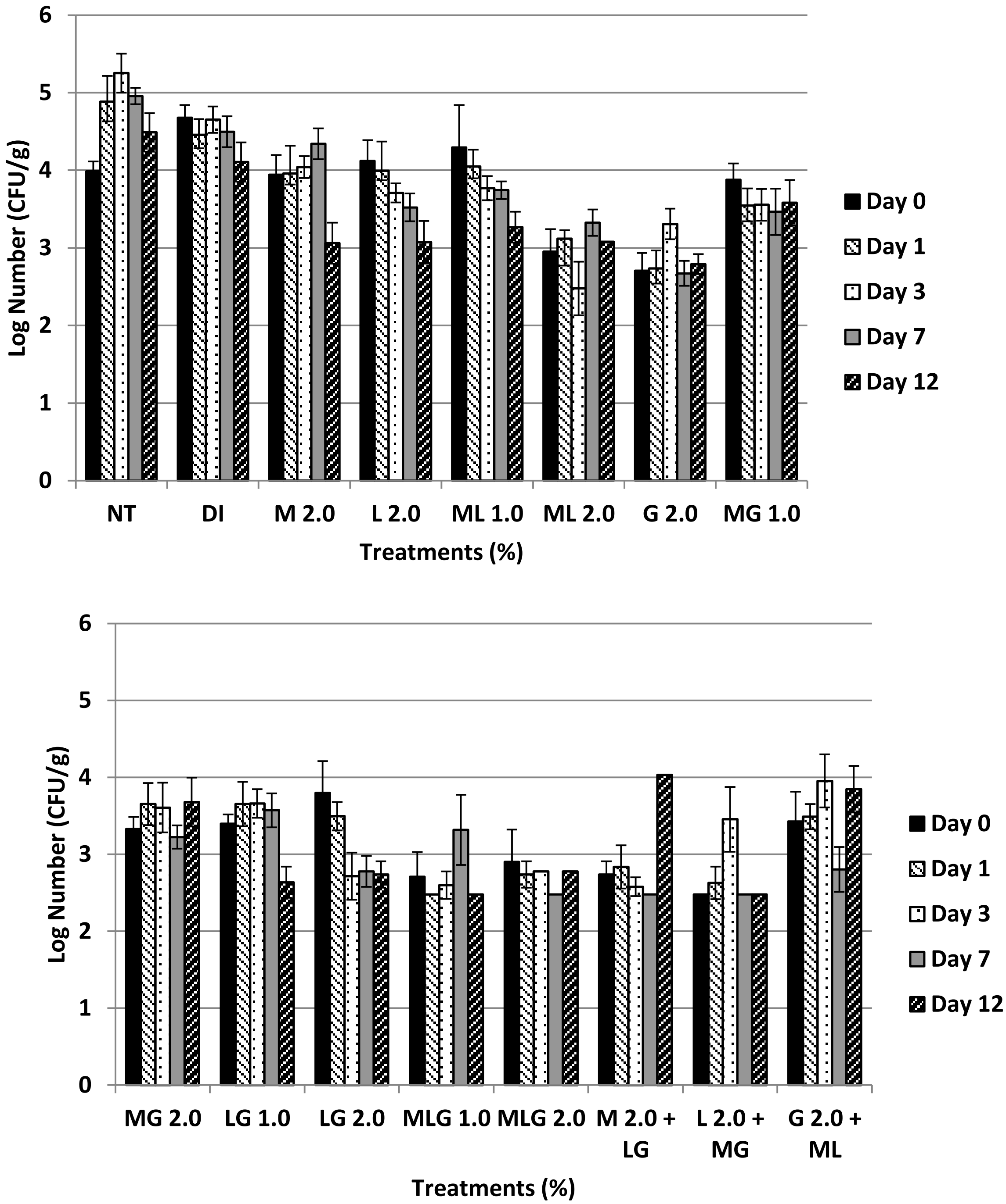


FIGURE 5: EFFECTS OF ORGANIC ACIDS AND GRAPE SEED EXTRACTS ELECTROSTATICALLY SPRAYED ON *SALMONELLA* TYPHIMURIUM INOCULATED TOMATOES FOR 12 DAY STORAGE PERIOD.

Values are means log numbers of eight replicates and error bars indicate the standard error of means. $P < 0.05$. Test treatments: NT (untreated tomatoes), DI (pH adjusted (2.0) deionized water), M (malic acid), L (lactic acid), G (grape seed extract).

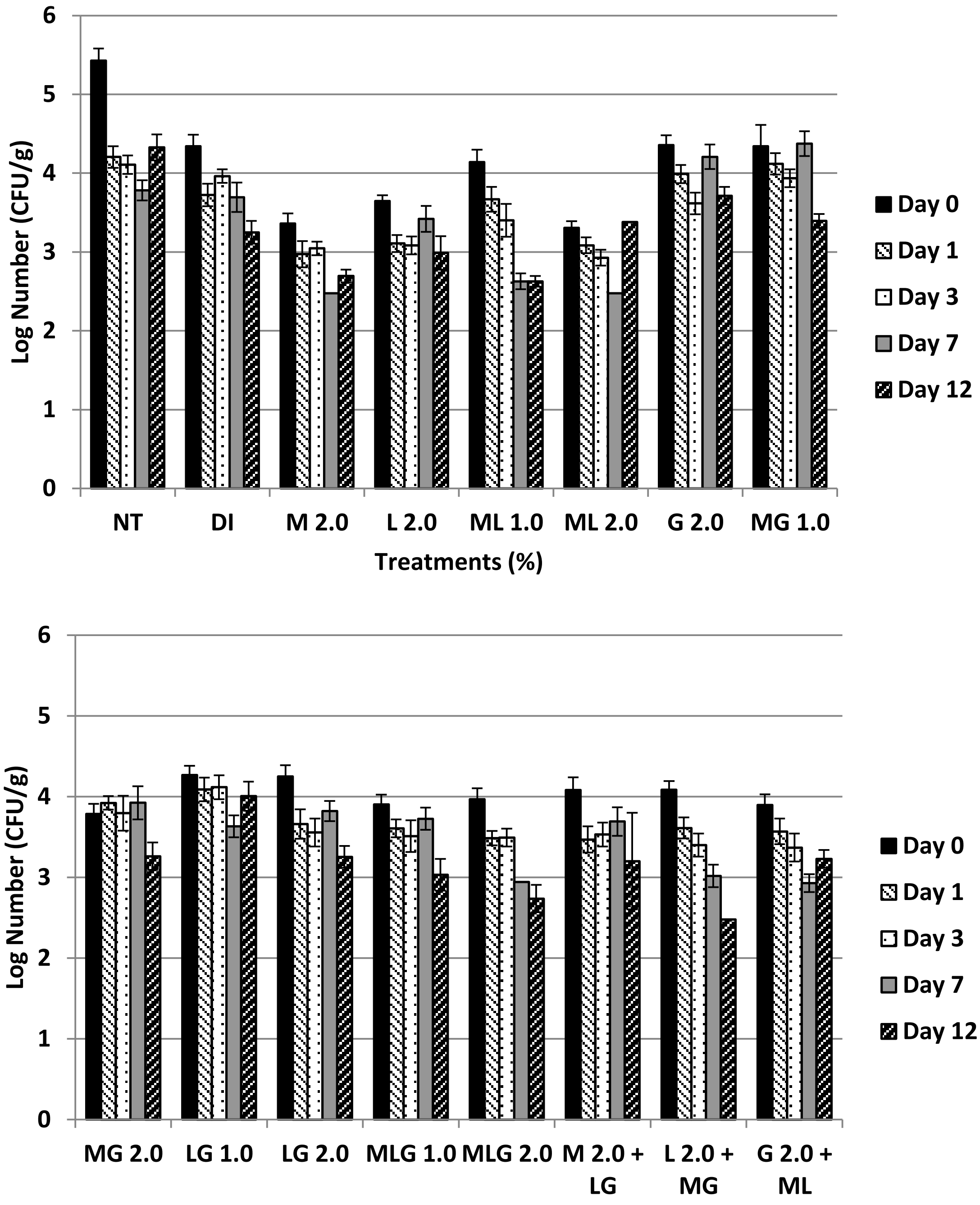


FIGURE 6: EFFECTS OF ORGANIC ACIDS AND GRAPE SEED EXTRACTS ELECTROSTATICALLY SPRAYED ON *E. coli* O157:H7 INOCULATED TOMATOES FOR 12 DAY STORAGE PERIOD.

Values are means log numbers of eight replicates and error bars indicate the standard error of means. $P < 0.05$. Test treatments: NT (untreated tomatoes), DI (pH adjusted (2.0) deionized water), M (malic acid), L (lactic acid), G (grape seed extract).

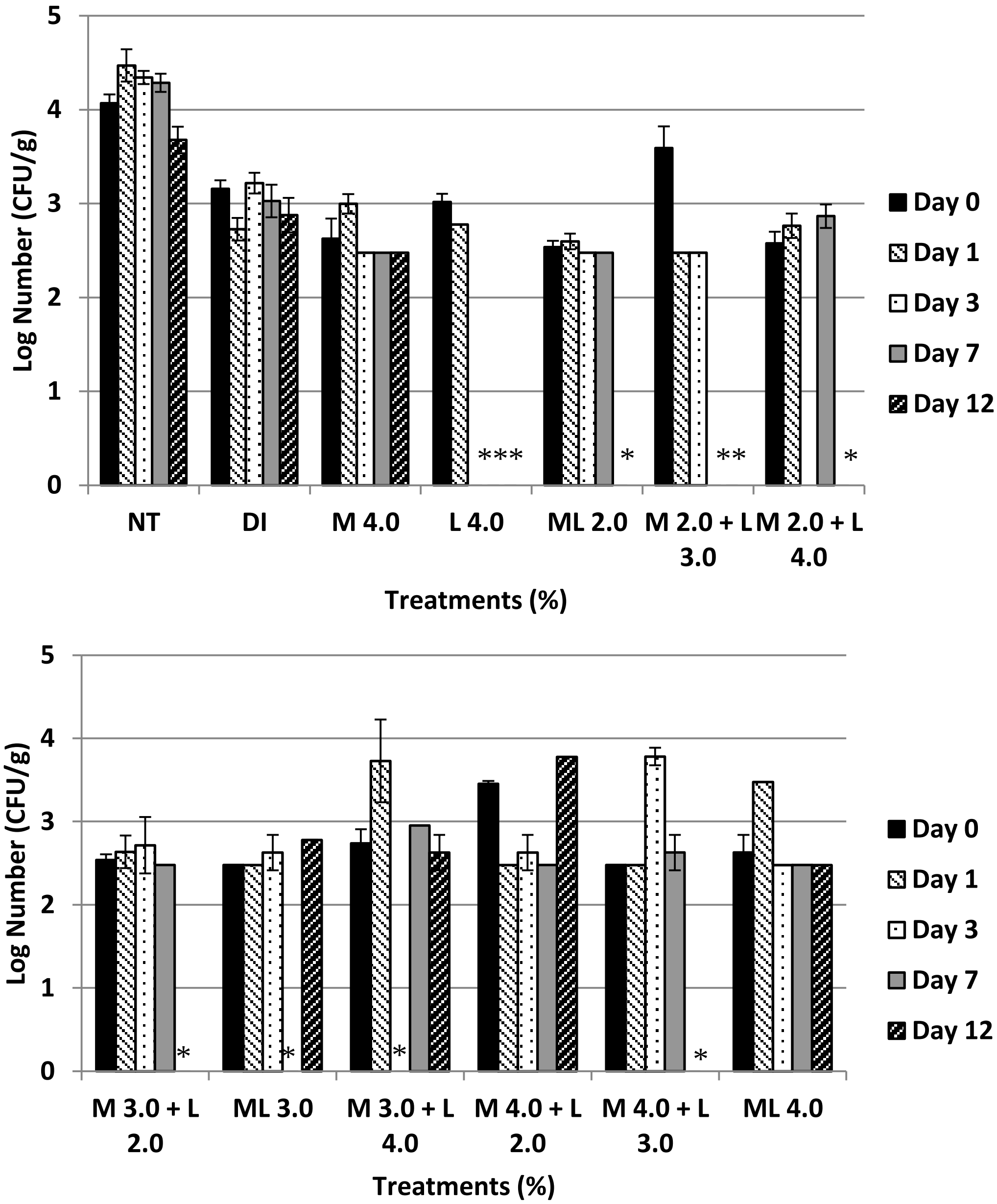
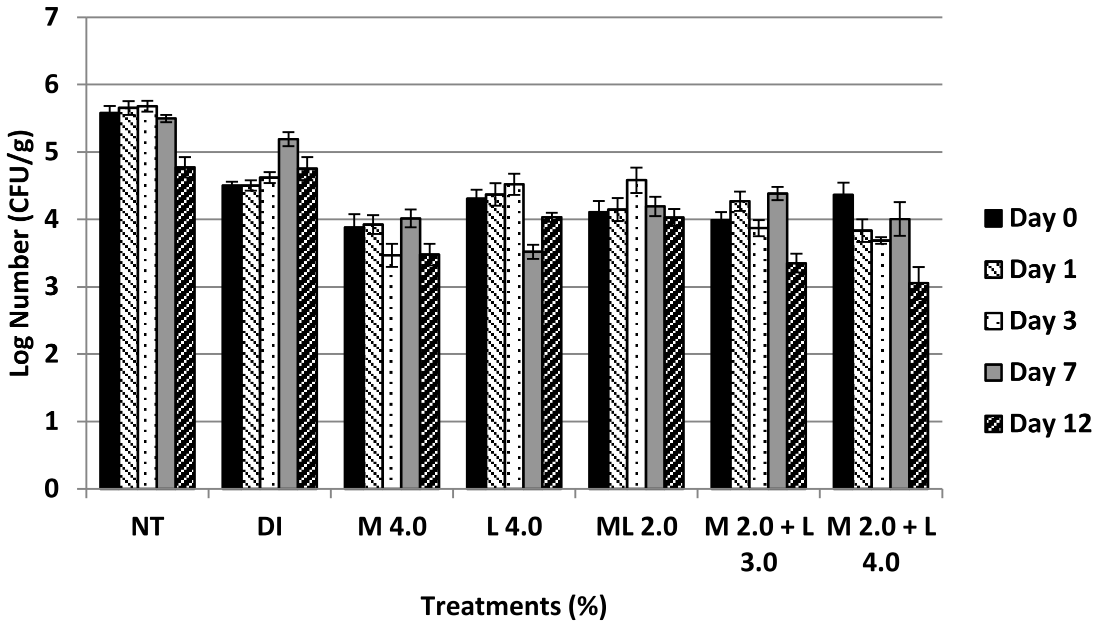


FIGURE 7: LOG NUMBER OF REMAINING *SALMONELLA* TYPHIMURIUM ON TOMATOES AFTER ELECTROSTATICALLY SPRAYED WITH ORGANIC ACIDS OVER 12 DAY STORAGE PERIOD.

Values are means log numbers of eight replicates and error bars indicate the standard error of means. $P < 0.05$.

* indicate below detection levels. Test treatments: NT (untreated tomatoes), DI (pH adjusted (1.9) deionized water), M (malic acid), L (lactic acid).



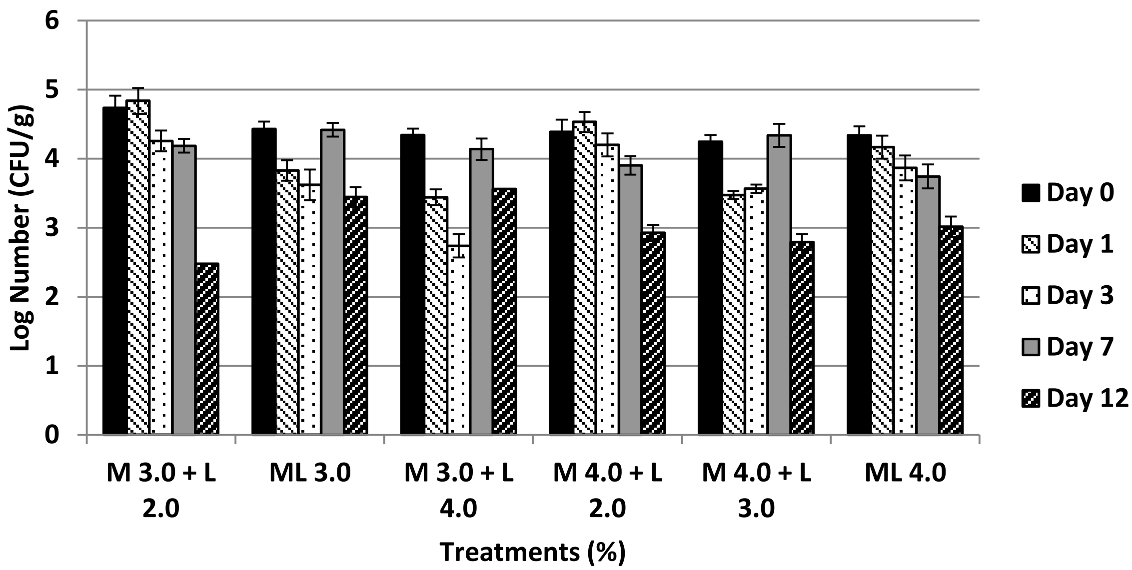


FIGURE 8: LOG NUMBER OF REMAINING *E. coli* O157:H7 ON TOMATOES AFTER ELECTROSTATICALLY SPRAYED WITH ORGANIC ACIDS OVER 12 DAY STORAGE PERIOD.

Values are means log numbers of eight replicates and error bars indicate the standard error of means. $P < 0.05$. Test treatments: NT (untreated tomatoes), DI (pH adjusted (1.9) deionized water), M (malic acid), L (lactic acid).

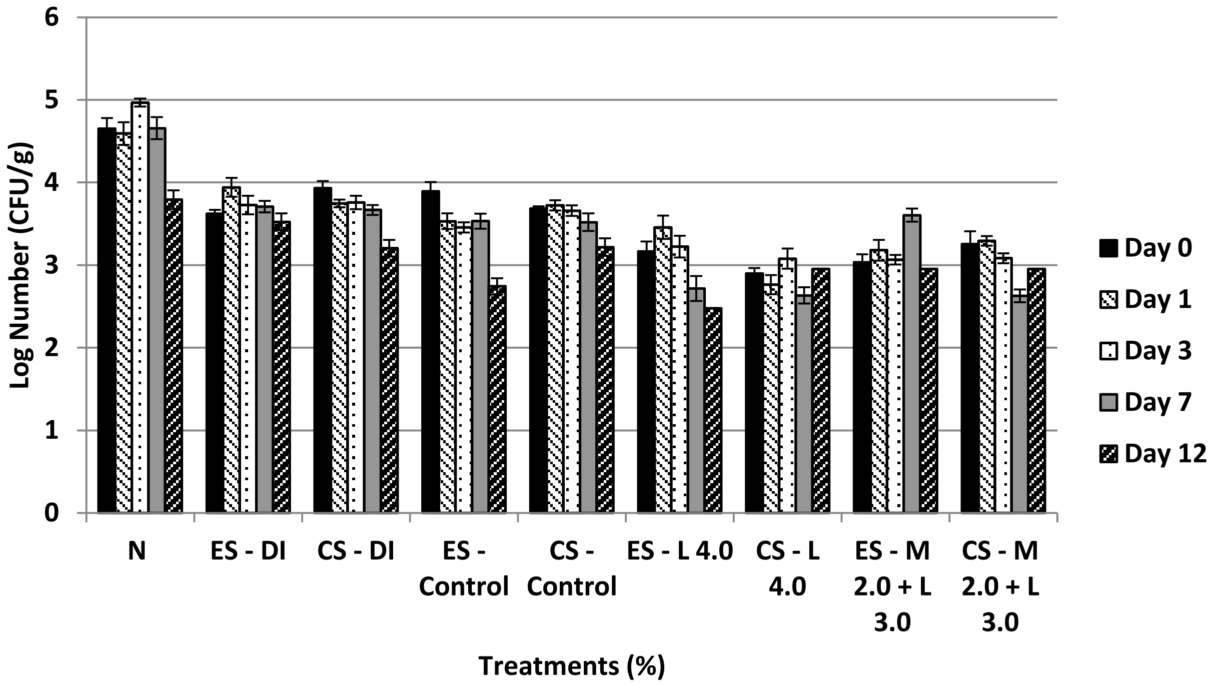


FIGURE 9: LOG REMAINING OF *SALMONELLA* TYPHIMURIUM ON TOMATOES SPRAYED WITH NATURAL ANTIMICROBIALS BY CONVENTIONAL AND ELECTROSTATIC SPRAYER.

Values are means log numbers of quadruplet analysis and error bars indicate the standard error of means. $P < 0.05$. Test treatments: ES (electrostatic sprayer), CS (conventional sprayer), NT (untreated tomatoes), Control (pH adjusted (1.9) deionized water), M (malic acid), L (lactic acid).

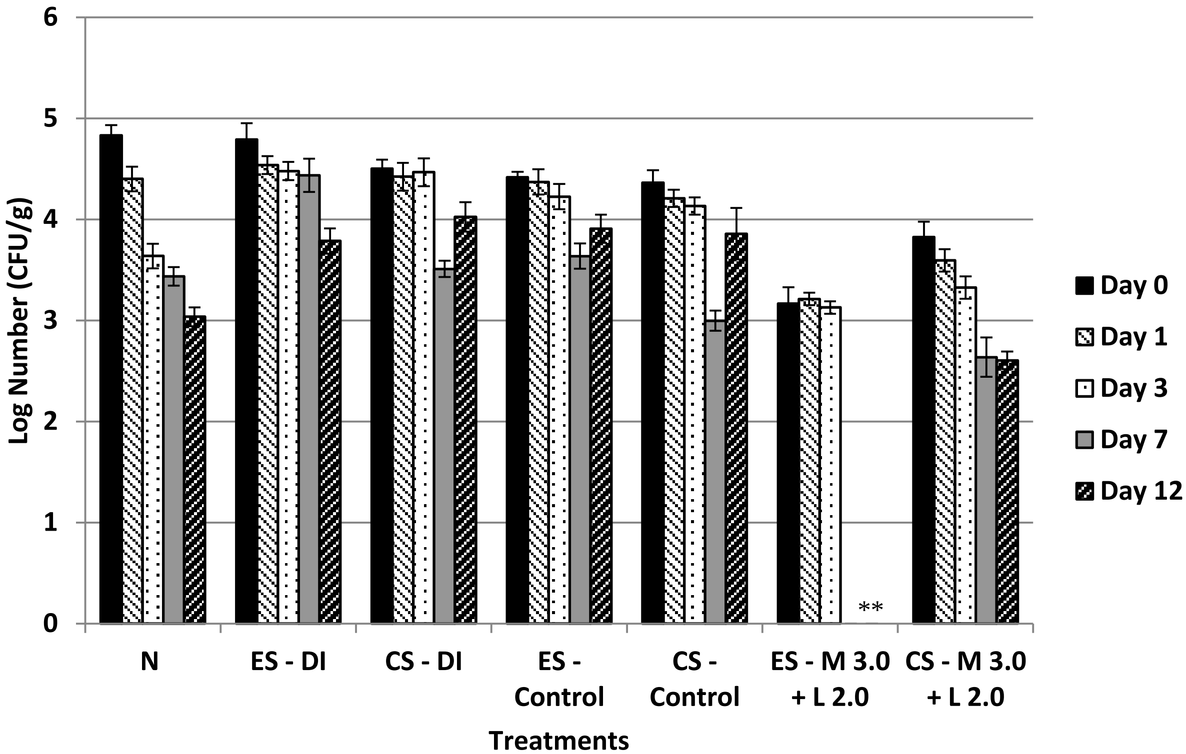


FIGURE 10: LOG REMAINING OF *E. coli* O157:H7 ON TOMATOES SPRAYED WITH NATURAL ANTIMICROBIALS BY CONVENTIONAL AND ELECTROSTATIC SPRAYER.

Values are means log numbers of quadruplet analysis and error bars indicate the standard error of means. * indicate below detection levels. P < 0.05. Test treatments: ES (electrostatic sprayer), CS (conventional sprayer), NT (untreated tomatoes), DI (sterile DI water), Control (pH adjusted (1.9) deionized water), M (malic acid), L (lactic acid).

Chapter 5: Investigate the effects of electrostatic spray containing optimized concentration of antimicrobials on the color and texture of uninoculated cantaloupe cubes and tomatoes.

Abstract

The consumption of fresh fruits and vegetables has led to an increase in foodborne illnesses. Produce industry has different methods of preventing contamination; however, these methods need to be improved. The application of organic acid by electrostatic spraying has been shown to be a potential strategy to reduce foodborne contamination. Yet, quality evaluations need to be performed to determine if any differences in fruits and vegetables would occur. The objective of this study was to determine the effect of the optimized concentrations of organic acids applied by electrostatic spray on cantaloupe and tomatoes. The concentrations and combinations of malic acid and lactic acid found to reduce foodborne pathogens in the previous (Chapter 3) were prepared and sprayed on the selected produce. Instrumental analysis of color and texture were performed on produce stored up to 12 days at 4°C. No significant differences in color were observed when comparing non-sprayed cantaloupe cubes and tomatoes when compared to sprayed samples. The texture of cantaloupe left untreated no difference between cantaloupe cubes treated with organic acids. Tomatoes sprayed with organic acids did not differ from untreated tomatoes until the 12th day of storage. Natural antimicrobials have the potential to be used in order to enhance the safety of fresh and fresh-cut produce without compromising the quality.

Introduction

The growth of the fresh fruit and vegetables industry is on the rise due to the demand by the consumers (Selma *et al.* 2008). Since the fruits and vegetables are minimally processed there is an increased risked of becoming contaminated with foodborne pathogens by cross-contamination from other produce or contaminated water in processing areas (Berger *et al.* 2010).The contaminated produce can cause foodborne illness.

Foodborne illnesses caused by like *Salmonella* and *E. coli* O157:H7 have caused over 20,000 illnesses annually when they have been reported. However many foodborne illnesses go unreported because some consumers may not know they have contracted foodborne illnesses and are not sick enough to go to the hospital (CDC 2011). Hurdle technology has been used to reduce the number of illnesses caused by foodborne pathogens. Hurdle technology is the use of multiple processes to improve the quality and safety of a product. Ukuku *et al.* (2006) used vacuum and steam treatments to improve the shelf life of whole and fresh-cut cantaloupe. Another study used hurdles of organic acids, hydrogen peroxide and mild heat to inactivate E. coli O157:H7 on baby spinach (Huang and Chen 2011).

Organic acids and plant extracts have been reported to reduce pathogen contamination on the surface of various fruits and vegetables. Akbas and Ölmez (2007) reported that lactic acid was most effective in reducing *Listeria monocytogenes* by approximately1.5 log CFU/g on the surface of lettuce. Another study treating spinach with a combination of organic acids and grape seed extract applied by an electrostatic sprayer was able to reduce *E. coli* O157:H7 by 4.0 CFU/g on the fourteenth day of observation (Ganesh *et al.* 2012).

The electrostatic sprayer has many advantages over immersion and conventional sprayers. This sprayer uses small droplets of solution with a charge that propels the particle to its target surface (Law and Cooper 2001). Studies have shown a reduction in treatment needed for application when compared to conventional sprayers (Kim and Hung 2007). The least amount of pesticide was used on strawberries when sprayed with an electrostatic sprayer than pesticide sprayed with conventional sprayer (Giles and Blewett 1991). Spraying organic acids and plant extract with an electrostatic sprayer may impart changes in the quality of the food. Therefore, quality attributes need to be evaluated to determine if there were any changes after treatment.

Quality attributes are very important when selling fresh fruit and vegetables. Color and texture are some of the most important quality attributes that consumers look for (Rosenfeld and Nes 2000). When antimicrobial are added to fresh produce, texture and color are evaluated to determine if there have been any qualities changes to the produce. Samara and Koutsoumanis (2009) reported that the color of lettuce did not change when organic acids were applied. Organic acid-incorporated soy protein coatings that were applied onto apples showed no difference in color or texture when compared to cantaloupe without soy protein coating (Eswaranandam *et al.* 2006). There is limited research on how quality attributes of tomatoes and cantaloupe cubes are affected after being treated with organic acids and plant extracts and needs to be investigated.

The objective of this study is to observe the effects of electrostatic spray containing optimized concentration of organic acids and plant extracts on the color and texture of uninoculated tomatoes and cantaloupe cubes.

Materials and Methods

Preparation of Samples

Cantaloupe and tomatoes were purchased from local grocery store. Cantaloupes were washed with water and cut into cubes (approximately 8 cm^3). Tomatoes were washed in water and dried at room temperature for 15 minutes on bench top surface. The prepared samples were sprayed electrostatically with optimized concentrations of test solutions (Table 1) determined from the previous chapter (Chapter 3) and the control (untreated). After spraying, the samples were allowed to dry for 30 min. The samples were package in individual plastic bags and stored at 4°C for 12 days.

Color Analysis

Color of cantaloupe cubes and tomatoes were measured using a chromameter (Minolta CR-300, Mintola Corporation, Ramsey, NJ, USA). Reading on four sides of the cantaloupe cube were measured and averaged. The tomatoes' color was measured in two different spots in the equatorial area. The L*, a*, and b* values were measured on 0, 1, 3, 7, 12 days after storage at 4°C.

Texture Analysis

The procedures followed were obtained from Eswaranadam *et al.* (2006) with following modifications. The texture of tomatoes and cantaloupe were measured by using Texture Analyzer (TA-XT2i, Texture Technologies Corp., Scarsdale, NY). Cylindrical samples were made from the middle portion of cantaloupe cubes. Both ends were trimmed making 10 mm samples. Samples were placed on flat plate attached to platform. A 3-inch diameter compression probe attached to load cell (5-kg) was used to compress the sample. The probe was set to move at a speed of 5 mm/s, test speed of 10 mm/s, and post speed of 10 mm/s. Hardness was measured by the maximum force (N) of the compression.

Whole cherry tomatoes were used. Tomatoes were placed on flat plate attached to a platform and a 3-inch diameter probe was attached to the load cell (5-kg). The compression probe was set to move at pre-speed at 5 mm/s, test speed 10 mm/s, and post-test speed at 10 mm/s. hardness again was measured by the maximum force (N) of the compression. Puncture strength was also measured. A 5-mm diameter cylindrical probe was attached to 5-kg load cell and the respective sample was placed on flat plate attached to platform. The maximum force at the point of puncturing was recorded at the puncture strength. Samples were tested on 0, 1, 3, 7, and 12 day of the trial.

Statistical Analysis

All results were analyzed in JMP (John's Macintosh Product) 10.0 software (SAS Inst. Inc., Cary, N.C., USA) using ANOVA (analysis of variance) and significant differences between results will be estimated at $P<0.05$.

Results and Discussion

Cantaloupe: Texture and Color

Natural antimicrobials solutions that reduced pathogen loads of *Salmonella* (3.6 log CFU/g) and *E. coli* (4.6 log CFU/g) were used to determine their effects on different quality attributes of cantaloupe cubes. As the storage period increased, the a* value (red-green coloring) increased indicating the cantaloupe was becoming more ripe (Table 2). Color values of the untreated samples were not significantly different from the sprayed samples except for M 4% + L 2% on day 12. L* value of cubes treated with combination of organic acids was significantly different from cantaloupe cubes untreated and treated with just malic acid (4%). The difference can be due to lack of polyphenol peroxidase which can induce darkening of flesh (Lamikanra *et al.* 2000). The texture of samples sprayed with M 4%+ L 2% was significantly different from the control on initial day of spraying (Table 3). The addition of multiple organic acids could increase the water loss, which can lead to loss of firmness. Also, lack of storage at low temperature could lead more softening of the tissues. Storage at low temperature is a preservation technique that helps to improve the quality of fresh produce after cutting (Jeong 2008). However, after being stored at 4°C for 12 days, firmness of cubes treated with M 4% and M 4% + L 2% did not exhibit more softening than the control (untreated cantaloupe cubes). On each day of measurement, the texture of the cantaloupe cubes treated with natural antimicrobials were not different from cubes left untreated. Other studies have reported similar results after using different technologies and antimicrobials on cantaloupe cubes. Fan *et al.* (2006) did not see any significant differences in firmness after whole melons were treated with hot water and the cubes cut from the melons treated with irradiation. Cantaloupe cubes coated with soy protein coating containing malic and lactic acid did not differ in firmness and color when measured over a 14 day storage period (Eswaranandam *et al.* 2006). Selma *et al.* (2008) found that cubes cut from whole cantaloupe melons treated and not treated with O_3 gas and/or hot water did not decrease over after being stored for 8 days at 5°C.

Tomatoes: Texture and color

Like the cantaloupe cubes, natural antimicrobial solutions that were found to reduce *E. coli* (2.3 log CFU/g) and *Salmonella* (undetectable levels) were sprayed onto tomatoes to determine if there were changes in texture and color over 12 day storage period. The a value indicates the redness of a color, and during the storage period there was no significant difference among the samples (Table 4). Similar results were seen in a study using different antimicrobials (chlorine, citric acid, UV light and ozone) applied to tomatoes (Bermúdez-Aguirre and Barbosa-Cánovas 2013). The firmness of tomatoes sprayed with antimicrobials did not exhibit any significant differences from tomatoes that were not sprayed until the 12th day of storage (Table 5 and 6). Tomatoes sprayed with combinations of malic and lactic acids were not as firm as other tomatoes in the study (untreated and treated with lactic alone). Also, a decrease in the firmness of tomatoes (untreated and treated) was observed as the storage period increased. Lu (2011) saw similar results of grape tomatoes (treated and untreated) during a 16 day storage period. The texture of tomatoes slices stored at 5°C as storage period (15 days) increased (Ayala-Zavala *et al.* 2008). This reduction in firmness could be due to enzymatic activity. During ripening, the cell wall breaks down which is caused by pectin-cleaving enzymes (Marín-Rodríguez *et al.* 2002). Degradation of the cell wall leads to liquefaction of the internal tissues causing the tomatoes to have reduced firmness.

Conclusion

Cut cantaloupe cubes and fresh tomatoes electrostatically sprayed with organic acids did not further decrease the texture or color for the 12 day storage period. The use of multiple hurdle technology has the potential to increase the safety of fresh fruits and vegetables without changes to quality attributes.

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Table 1: Optimized Concentrations of Natural Antimicrobials Sprayed on Cantaloupe Cube and Tomatoes

	Treatments	
	M[a] (%)	**L[b] (%)**
Cantaloupe Cubes	4	0
	4	2
	Control[c]	
Tomatoes	0	4
	2	3
	3	2
	Control	

[a]M (malic acid), [b]L (lactic acid**),** [c]Control (no organic acid treatment)

Table 2: Color of Cantaloupe Cubes Electrostatically Sprayed with Organic Acids for Day 0, 1, 3, 7, and 12.

		Color		
Day	Treatments	L	a	b
0	[a]Control	[b]63.5 ± 0.24a	9.6 ± 0.29a	29.2 ± 0.02a
	M 4%	64.2 ± 0.39a	8.9 ± 0.12a	30.0 ± 0.27a
	M 4 % + L 2%	65.3 ± 0.79a	9.4 ± 0.21a	30.6 ± 0.67a
1	Control	64.3 ± 0.36a	9.6 ± 0.16a	28.9 ± 0.32a
	M 4%	64.3 ± 0.21a	9.5 ± 0.09a	29.7 ± 0.43a
	M 4 % + L 2%	64.1 ± 0.35a	9.4 ± 0.13a	30.8 ± 0.49a
3	Control	63.3 ± 0.32a	9.8 ± 0.07a	29.9 ± 0.13a
	M 4%	63.5 ± 0.27a	9.2 ± 0.13a	30.8 ± 0.33a
	M 4 % + L 2%	65.5 ± 0.76a	9.1 ± 0.23a	30.1 ± 0.40a
7	Control	62.7 ± 0.41a	9.9 ± 0.23a	29.9 ± 0.32a
	M 4%	63.9 ± 0.20a	9.6 ± 0.02a	29.7 ± 0.29a
	M 4 % + L 2%	64.2 ± 0.45a	9.1 ± 0.17a	30.0 ± 0.48a
12	Control	62.4 ± 0.66a	9.9 ± 0.23a	29.5 ± 0.45a
	M 4%	63.2 ± 0.40a	9.6 ± 0.02a	30.1 ± 0.16a
	M 4 % + L 2%	64.9 ± 0.49b	9.1 ± 0.17a	30.9 ± 0.32a

[a]Control (no organic acid treatment), M (malic acid), L (lactic acid).
[b]Values are the mean of L, a, b values ± SEM
Means following the same letters in the column within the same day are not significantly different (P > 0.05).

Table 3: Texture of Cantaloupe Cubes Electrostatically Sprayed with Organic Acids for Day 0, 1, 3 7, and 12.

Treatments	**Texture (N)**				
	Day 0	Day 1	Day 3	Day 7	Day 12
Control[a]	30.0 ± 0.84a[b]	30.2 ± 0.47ab	31.7 ± 0.48a	34.8 ± 0.79a	30.6 ± 0.71a
M 4%	34.0 ± 0.58a	26.7 ± 0.41b	28.7 ± 0.33a	30.9 ± 0.45a	33.4 ± 0.68a
M 4% + L 2%	25.7 ± 0.33b	32.8 ± 0.21a	29.3 ± 0.54a	33.9 ± 0.41a	33.8 ± 0.81a

[a]Control (no organic acid treatment), M (malic acid), L (lactic acid).
[b]Mean Force (N) ± SEM.
Means following the same letters in the columns are not significantly different (P > 0.05).

Table 4: Color of Tomatoes Electrostatically Sprayed with Organic Acids for Day 0, 1, 3, 7, and 12.

		Color		
Day	Treatment	L	a	b
0	Control[a]	43.0 ± 0.33a[b]	11.4 ± 0.27a	16.2 ± 0.06a
	L 4%	41.7 ± 0.34a	11.6 ± 0.25a	15.4 ± 0.29a
	M 2% + L 3%	41.2 ± 0.20a	10.8 ± 0.19a	14.2± 030a
	M 3% + L 2%	42.6 ± 0.26a	9.8 ± 0.43a	15.2 ± 0.20a
1	Control	43.7 ± 0.57ab	12.1 ± 0.28a	15.0 ± 0.52a
	L 4%	40.4 ± 0.45c	12.2 ± 0.31a	17.3 ± 0.50ab
	M 2% + L 3%	40.5 ± 0.05bc	12.6 ± 0.40a	17.6 ± 0.11ab
	M 3% + L 2%	43.5 ± 0.37ab	13.9 ± 0.23a	17.8 ± 0.06b
3	Control	44.5 ± 0.63a	10.2 ± 0.22a	12.5 ± 0.17b
	L 4%	43.0 ± 0.25a	11.7 ± 0.55a	14.0 ± 0.48ab
	M 2% + L 3%	42.2 ± 0.37a	10.5 ± 0.17a	13.6 ± 0.13ab
	M 3% + L 2%	41.6 ± 0.37a	10.7 ± 0.14a	13.9 ± 0.23ab
7	Control	41.1 ± 0.24ab	12.7 ± 0.22a	11.9 ± 0.27ab
	L 4%	43.2 ± 0.21a	11.4 ± 0.19a	11.0 ± 0.24b
	M 2% + L 3%	42.4 ± 1.4a	11.9 ± 0.40a	10.1 ± 0.10b
	M 3% + L 2%	38.2 ± 0.15b	11.2 ± 0.10a	13.4 ± 0.15a
12	Control	42.0 ± 0.04a	11.9 ± 0.32ab	14.8 ± 0.52b
	L 4%	42.2 ± 1.50a	11.7 ± 0.47ab	14.9 ± 0.83b
	M 2% + L 3%	45.3 ± 0.82a	11.1 ± 0.26b	14.8 ± 0.13b
	M 3% + L 2%	44.6 ± 0.87a	12.9 ± 0.22a	19.7 ± 0.61a

[a]Control (no organic acid treatment), M (malic acid), L (lactic acid).
[b]Values are the mean of L, a, b values ± SEM
Means following the same letters in the column within the same day are not significantly different (P > 0.05).

Table 5: Texture Analysis of Compression of Tomatoes Electrostatically Sprayed with Organic Acids for Day 0, 1, 3, 7, and 12.

Treatments	**Texture (N)**				
	Day 0	Day 1	Day 3	Day 7	Day 12
Control[a]	32.8 ± 0.39a[b]	34.3 ± 0.89ab	25.7 ± 0.37a	29.8 ± 0.26a	21.3 ± 0.33bc
L 4%	30.9 ± 0.28a	34.6 ± 0.46a	27.3 ± 0.47a	28.4 ± 0.42a	20.2 ± 0.44c
M 3% + L 2%	32.2 ± 0.98a	30.6 ± 0.13b	27.5 ± 0.31a	27.1 ± 0.57a	23.0 ± 0.44ab
M 2% + L 3%	31.3 ± 0.34a	30.8 ± 0.18b	25.7 ± 0.54a	29.2 ± 0.24a	24.7 ± 0.34a

[a]Control (no organic acid treatment), M (malic acid), L (lactic acid).
[b]Mean Compression Force (N) ± SEM.
Means following the same letters in the columns are not significantly different (P > 0.05).

Table 6: Texture Analysis of Puncture of Tomatoes Electrostatically Sprayed with Organic Acids for Day 0, 1, 3 7, and 12.

Treatments	Texture (N)				
	Day 0	Day 1	Day 3	Day 7	Day 12
Control[a]	16.0 ± 0.18a[b]	15.0 ± 0.16a	15.3 ± 0.18a	14.1 ± 0.26ab	14.3 ± 0.32a
L 4%	15.1 ± 0.23a	15.9 ± 0.33a	15.9 ± 0.20a	14.7 ± 0.25a	14.9 ± 0.22a
M 3% + L 2%	15.4 ± 0.21a	15.5 ± 0.13a	14.6 ± 0.45a	12.3 ± 0.25b	10.5 ± 0.11b
M 2% + L 3%	16.8 ± 0.24a	15.6 ± 0.25a	14.6 ± 0.16a	15.6 ± 0.43a	14.7 ± 0.48a

[a]Control (no organic acid treatment), M (malic acid), L (lactic acid).
[b]Mean Puncture Force (N) ± SEM.
Means following the same letters in the columns are not significantly different ($P > 0.05$).

OVERALL CONCLUSION

Organic acids and grape seed extract were able to reduce foodborne pathogen on various fresh and fresh-cut produce. However, malic and lactic acids exhibited more antimicrobial activity than grape seed extract. The different combinations and concentrations of the organic acids were able to reduce foodborne pathogens (*Salmonella* Typhimurium and *Escherichia coli* O157:H7) on cantaloupe cubes by 3.6 and 4.6 log CFU/g and on tomatoes by ≥ 3.7 and 2.3 log CFU/g when stored at 4ºC for 12 days. Electrostatically spraying the antimicrobials has demonstrated more log reduction of microbes when compared to conventional spraying applications. The natural antimicrobials applied with the novel technology were able to decrease microbial load without major changes to texture and color of tested produce. Increasing food safety in the fresh produce industry can be possible with the use of natural antimicrobials applied by the electrostatic sprayer.